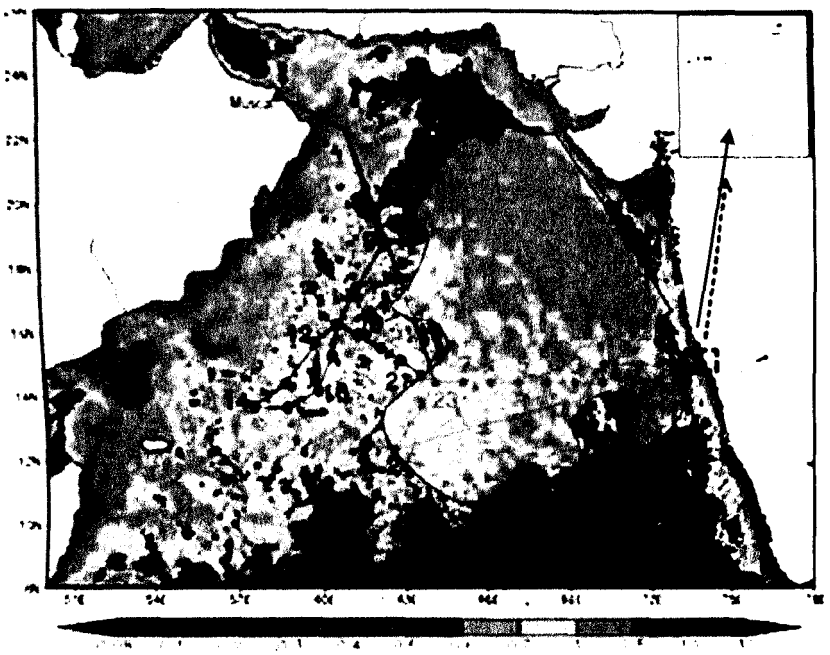
EFFECT OF OXYGEN DEFICIENCY ON PRODUCTIVITY AND PLANKTON COMPOSITION IN THE ARABIAN SEA



Thesis Submitted To
GOA UNIVERSITY
For the degree of

DOCTOR OF PHILOSOPHY
In The Faculty of
ZOOLOGY

By

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December, 2012

CERTIFICATE

This is to certify that **Ms. Sunita Shantaram Mochemadkar** has duly completed the thesis entitled **"EFFECT OF OXYGEN DEFICIENCY ON PRODUCTIVITY AND PLANKTON COMPOSITION IN THE ARABIAN SEA"** under my supervision for the award of the degree of Doctor of Philosophy.

This thesis being submitted to the Goa University, Taleigao Plateau, Goa for the award of the degree of Doctor of Philosophy in Zoology is based on original studies carried out by her

The thesis or any part thereof has not been previously submitted for any other degree or diploma in any Universities or Institutions.

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Place: Goa Univ

Certified that the student has incorporated all revision/modification/correction if any suggested by the examiners

8/8/2013

DECLARATION

As required under the University Ordinance 0.19.8 (iv), I hereby declare that the present thesis entitled **"EFFECT OF OXYGEN DEFICIENCY ON PRODUCTIVITY AND PLANKTON COMPOSITION IN THE ARABIAN SEA"** is my original work carried out and the same has not been submitted for any other degree or diploma. To the best of my knowledge, the present research is the first comprehensive work of its kind from the area studied.

SUNITA S. MOCHEMADKAR

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SUNITA S. MOCHEMADKAR

PREFACE

The marine food web is largely dominated by organisms called 'plankton' that are hardly visible to the human eye and one need to use microscopes to realize their diversity and abundance. Phytoplankton (plants of the sea) that constitutes primary production is utilized by organisms called zooplankton (animals of the sea). Thus, latter organisms constitute a very important link between small and large organisms and hold a central position in the marine food web.

Mesozooplankton (>200μm) are the diverse and delicate heterotrophic organisms that include a great majority of taxa of invertebrates, which drift in the waters of the world's oceans. Though ubiquitous in freshwater to marine ecosystems, their importance in reduced dissolved oxygen in the water column of the Arabian Sea is not much known. Zones of low oxygen in the water column are known to occur throughout the world's oceans. And, Arabian Sea is one of the region that harbor both permanent and seasonal low oxygen waters (0_2< 0.1ml/l). There is a growing concern that hypoxic and anoxic waters in the sea spread in extent and intensity, posing a severe risk to marine aquatic life. In fact, the studies on mezooplankton in recent times show that most mesozooplankton are generally not found in the water column where dissolved oxygen concentrations falls below certain critical level, though some species are able to thrive even below this critical level indicating that mesozooplankton has a variety of roles in the pelagic even in reduced environment. Thus studying their ecology, abundances and relationship with other planktonic organisms is ultimately important in understanding the trophic organisation in pelagic ecosystems, and carbon turnover.

There have been no serious attempts to characterise plankton (phyto- and zoo) community composition of reduced environment prevailing in the water column of this sea in understanding their role in the food web. The resulting account on mesozooplankton (and phytoplankton) from the eastern Arabian Sea in this thesis is one of the first of its kind from the tropical waters experiencing permanent and seasonal oxygen minimum zones (OMZ). While there are some studies on distributions of these forms from the open waters OMZs of the Arabian Sea.

Vagaries of the monsoons certainly make the northern Indian Ocean a very untypical area among the world oceans. The Arabian Sea is particularly intriguing with its mysteries only slowly unfolding. The oxygen minimum zone that occurs in the Arabian Sea remains enigmatic with regard to zooplankton and phytoplankton. By having carefully analyses the distribution, abundance and types of mesozooplankton and phytoplankton in water column experiencing reduced dissolved oxygen, coastal and open ocean regions of the Arabian Sea, I hope to provide certain new insights on this interesting, composite key component of plankton life at sea. Previous studies from the West coast of India indicate that the distributions of zooplankton taxa were influenced by season, depth of the sampling station and prevailing hydrographic conditions. Therefore, I have also related the plankton data with physics, chemistry and the general biology from the study area with a view to provide a scenario on the interplay of ecosystem dynamics on phyto- and zoo-plankton.

This thesis is written as part of the fulfilment of a Ph.D. from Goa University, Goa. The understanding of marine ecology of western continental shelf of India is still a challenging subject. I will find it interesting to follow this work to understand the importance and behaviour of copepod-zooplankton in shelf systems in the future.

The frontispiece depicts the study area viz. coastal and open waters of the northern Arabian Sea.

DEDICATED

TO MY

BELOVED PARENTS

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CHAPTER 1

INTRODUCTION

1.1: PLANKTON

Plankton includes organisms that passively drift, maintained in suspension by water current, or float or swim weakly comprise the plankton. They include photosynthetic phytoplankton, heterotrophic bacterioplankton, and zooplankton.

The science of studying the life and activity of plankton is called planktology. The word 'Plankton' comes from the Greek word "planktos" which means drifting; it was first coined by the Greek founder Victor Hensen (1887). Based upon the size of plankton, Dussart (1965) classified plankton as ultraplankton (0.2-2μm), nanoplankton (2-20μm), microplankton (20-200μm), mesoplankton (200μm-2mm) and megaplankton (>2mm). However, planktology is also concerned with taxonomy, the variation of species composition, abundance and distribution in relation to the physical and chemical factors of marine environment. They are important as current indicators, pelagic sediments on the sea floor and biological processes involved in the sea-air interaction, together with the trophic dynamics of the marine ecosystem.

Plankton being the foundation of the ocean food web plays an important role in the biogeochemical cycling of many important elements particularly carbon "Biological pump" (Fig. 1). They play an important role in the biogeochemical cycling of many important elements particularly carbon "Biological pump" (Fig. 1). Biological processes affect transport of organic carbon into the oceans' interior which in turn affects atmospheric CO_2. The annual uptake of CO_2 by the surface ocean varies between 1-3 Gt carbon (Battle *et al.*, 2000). It is estimated that only 2% human food originates from the ocean but contributes to 20% of high protein nutrition.

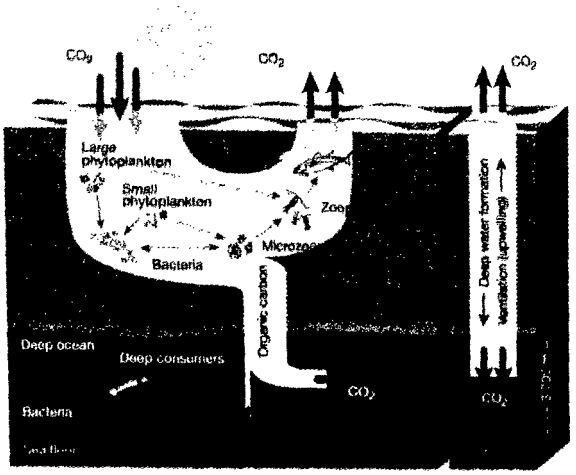


Fig. 1.1. The 'biological pump' depicting complex phytoplankton-based food web (taken from S. W. Chisholm; Nature 407, 685-687, 2000).

The composition and quantity of the plankton varies seasonally and from year to year and the success of fish stocks can depend on the plankton being in the right place at the right time. This plankton are very important to the ocean and to the whole planet. Further, long-term records indicate that plankton abundance and species composition may change substantially over decadal time scales (Lalli and Parsons, 1997). Decreasing plankton biomass may be caused by climate changes that increase water stratification and depress upwelling; conversely, in other regions, increasing winds may enhance nutrient concentrations in the euphotic zone and lead to increased phytoplankton and zooplankton production. They being at the base of the trophic pyramid, plays a fundamental role in marine food-webs. Therefore, any change in plankton will have consequences on the marine food-web and on other trophic levels through bottom-up control.

1.1.1: PHYTOPLANKTON

Phytoplankton are the autotrophic component of plankton with pigment or chromatophore like chlorophyll or the presence of accessory pigments such as phycobiliproteins.

1.1.1: PHYTOPLANKTON

Phytoplankton are the autotrophic component of plankton with pigment or chromatophore like chlorophyll or the presence of accessory pigments such as phycobiliproteins, xanthophylls etc. Due to their pigment, they preferentially absorb the red and blue portions of the light spectrum (400-700nm) for photosynthesis and reflect green light. Phytoplankton occur as unicellular, colonial or filamentous forms that live in the euphotic zone of an ocean, sea, lake or other body of water including under ice in polar areas. Like terrestrial plants, they convert inorganic materials (e.g. nitrate, phosphate, silicate) into new organic compounds (carbohydrates, lipids, proteins) with the help of light and atmospheric CO_2 by the process called "photosynthesis" and must therefore live in the well-lit surface layer. Since phytoplankton use atmospheric CO_2 to produce carbohydrates source of energy, they are known as the "primary producers" and generate approximately 70% of the oxygen in the Earth's atmosphere. There are more than 40,000 different species or strains of phytoplankton that scientists have classified in our oceans today. One quarter of all vegetation on planet Earth (both land and sea) consists of marine phytoplankton. They are composed of various groups *viz. Bacillariophyta, Pyrrophyta, Chlorophyta, Cyanophyta, Chrysophyta, Xanthophyta, Cryptophyta* and *Euglenophyta* of which *Bacillariophyta* (Diatom) and *Pyrrophyta* (dinoflagellate) are the most important.

Although oceans cover about 71% of the earth surface area, a major fraction of global primary production is of terrestrial origin. In total, the primary productivity of the world ocean is about $4x10^9$ tonnes of carbon per year (Lalli and Parson, 1997). The ocean contributes nearly 25-50% of the global primary production of which more than 90% is produced by phytoplankton and benthic macro algae and less than 10% by marshes. They are responsible for approximately half of the planet's total annual photosynthetic production. The amount of plant tissue build up by photosynthesis over time period is referred as primary productivity. Even in ideal conditions an individual phytoplankton only lives for about a day or two. When it dies, it sinks to the bottom. Consequently, over geological time scale, the ocean has become the primary storage sink for atmospheric carbon dioxide. Globally about 90% of photosynthetically fixed carbon sinks to the bottom of the ocean and is deposited primarily in the form of dead biomass.

The appearance of phytoplankton production and distribution depends on the availability of light, temperature, nutrients, buoyancy regulation and incidence of grazing. Light, temperature and nutrients are the primary factors regulating phytoplankton growth. Most of the phytoplankton are denser than water yet regulate buoyancy. Some have flagella, whose movement may counter the tendency to sink. Non-flagellated types have evolved cell or colony shapes that decrease the rate of sinking. They satisfy their carbon and energy needs through photosynthesis.

The relative availability of nutrients for phytoplankton can be used to classify aquatic environments. Regions having low concentration of essential nutrients and therefore low productivity, are called oligotrophic, and have chlorophyll concentration 0.05-0.5μg L^{-1}. Eutrophic water contains nutrients in high concentration ranging from 1-10μg L^{-1} while mesotrophic is a term applied to waters of intermediate chlorophyll concentration. The phytoplankton community of an oligotrophic lake is likely to be of small sized organisms with high surface to volume ratios. Eutrophic waters may be able to sustain greater proportions of larger sized organisms. A phytoplankton bloom develops when a species suddenly increases greatly in numbers under favourable conditions and gives a coloured appearance to the water. Some red tide species of *Alexandrium, Pyrodinium* and *Gymnodinium* are common in the coastal waters, which produce a variety of neurotoxins and hepatotoxins collectively referred to as saxitoxin (cyclic polypeptides), is lethal to life. There is increasing evidence that these toxins may be passed into food webs and hence may have widespread adverse effects.

Since phytoplankton depend upon certain conditions for growth, they are a good indicator of change in their environment. Phytoplankton respond very rapidly to environmental changes and can double its numbers on the order of once per day. In early 1930's, the potential role of iron as a limiting factor in phytoplankton productivity was appreciated (Gran, 1931; Hart, 1934; Harvey, 1938). The iron fertilization hypothesis postulated by John Martin in the year 1990 advocated the use of iron, to enhance oceanic primary production in High Nutrient Low Chlorophyll (HNLC) regions. The HNLC regions of the subarctic Pacific, the Southern ocean around Antarctica, and the equatorial Pacific (Cullen, 1991), make up about 20% of the total area of the ocean. Through iron enrichment, phytoplankton would be able to sequester carbon dioxide

out of the atmosphere into the ocean and act as a sink for fossil fuel carbon dioxide and thereby perhaps help to reduce global warming (Martin, 1990).

In the last decade, flow cytometry (FCM) has been increasingly used to analyze natural communities of marine microorganisms. The FCM provides rapid and accurate measurements of individual phytoplanktonic cells that are too dim to be discriminated by epifluorescence microscopy, which suggests greater dominance of smallest size class of the plankton called "Picoplankton" (0.2-2μm), (*Prochlorococcus*, *Synechococcus* and *Picoeucaryotes*). Picoplankton collectively are responsible for the most primary productivity in oligotrophic gyres (Azam *et al.*,1983; Li *et al.*, 1992; Campbell *et al.*, 1994). *Prochlorococcus* (Chisholm *et al.*, 1988) are known to inhabit in tropical and temperate oceans of the world (Chisholm *et al.*,1992) and is more common offshore, in waters of low nitrate compared to diatoms (Shalapyonok *et al.*, 2001).While, *Synechococcus* (Waterbury *et al.*, 1979) they are quite ubiquist, but most abundant in relatively meso-oligotrophic waters. The northern Arabian Sea during SWM are characterized by dominance of *Synechococcus* and eukaryotic picophytoplankton.

1.1.2: ZOOPLANKTON

Zooplankton are the diverse, delicate and often very beautiful, assemblage of animals that drift the waters of the world's oceans. They are more complicated in species composition and include a great majority of taxa of invertebrate, from the lowest protozoa to higher Urochordata. They can be found in the sun-lit zone and in deep ocean waters. Zooplankton range in size from tiny microbes to jellyfish. Within the plankton, holoplankton are those organisms that spend their entire life cycle as part of the plankton (e.g. most algae, copepods, salps, and some jellyfish). By contrast, meroplankton are the ones that are only planktonic for part of their lives usually the larval stage, and then graduate to either the nekton (free-swimming) or a benthic (sea floor) existence. Examples of meroplankton include the larvae of sea urchins, starfish, crustaceans, marine worms, and most fish. In species composition, zooplankton includes Protozoa, Coelenterata, Ctenophores, Crustacea, Gastropoda, Chaetognatha, Tunicata and planktonic larvae. Apart from this, also includes benthic taxa such as Polycheata.

Overall, the numbers of species of epipelagic and mesopelagic zooplankton are higher in low latitudes, but the numbers of individuals tend to be relatively low. The reverse situation is found in the higher latitudes, where there are fewer species but with higher abundance.

The most important group pertaining to this study is Copepoda (Supercalss Crustacea). The subclass Copepoda consists of 10 orders namely, *Calanoida, Cyclopoida, Poecilostmatoida, Harpacticoida, Siphonostomatoida, Monstrilloida, Misophrioida, Mormoniiloida, Platycopioida* and *Gelyelloida*. They exhibit great diversity in morphology, feeding behavior as well as the habitat occurring in marine, estuarine and fresh water areas. They also live in interstitial, subterranean and deep sea hydrothermal vents. According to the author Madhupratap (1999) there are 11500 known species belonging to 198 families and 1600 genera in the Indian Ocean. About one third of marine copepods are parasitic, or associated with invertebrate hosts, they belong to the orders *Monstrilloida, Poecilostomatoida* and *Siphonostomatoida* and few species of *Cyclopoida* and *Harpacticoida*. Whereas, species belonging to the families *Calanoida, Platycopioida, Gelyelloida, Mormonilloida, Misophrioida* and few species of *Poecilostomatoida* are free-living. Of all, Calanoids are the most common and abundant forms in the world ocean. Misophrioids are known to exist in deep sea hydrothermal vents, however till date, no such records are reported from the Arabian Sea.

Zooplankton are of ecological and economic significance, some forms of plankton are capable of independent movement and can swim up to several hundreds of meters vertically in a single day (a behavior called diel vertical migration), their horizontal position is primarily determined by currents in the body of water they inhabit. They contribute to deep scattering layer caused by the aggregation of animals which are detected by sonar tracers. Some deep animals who are capable of producing and emitting light known as bioluminescence which acts as warning signals (defence mechanism) to predators, for mating e.g. few medusae, Ctenophores, Siphanophore, Ostracods and Euphausids. Further, certain organisms are capable of concentrating radio-isotopes as indicators of pollutants, the study of which is important to marine environmental projects.

Zooplankton play a key role in the pelagic food web as it transfers the organic energy to the higher trophic levels such as carnivores (Santhakumari and Peter, 1993) and pelagic fish stock which is then exploited by man. Thus, controlling phytoplankton production and shaping pelagic ecosystems. Its importance lies in the fact that it constitutes the main food of many economically important animals including baleen whales and fishes (herring, mackerel, sardine etc, especially during young stages). During the feeding season, these fish migrate in schools to the feeding ground rich in food such as various groups of crustaceans like copepods and Euphausids which can be used as indicators for finding the migratory route and fishing grounds. It is this role, which has made zooplankton ecology of particular interest to The International Council for the Exploration of the Sea established in 1902. The International Indian Ocean Expedition (IIOE) (Zeitzschel, 1973) provides data on zooplankton and fish distributions over the entire Indian Ocean.

Zooplankton grazing also largely determines the amount and composition of vertical particle flux (Nair *et al.*, 1989; Hakke *et al.*, 1993) This not only fuels the benthos community but contributes to the removal of surplus anthropogenic CO_2 from the atmosphere through sedimentation and burial of organic and inorganic carbon compounds. It is thus important to increase our comparatively spare knowledge of all aspects of plankton ecology by a joint effort on the basis of intercomparable methods understanding and predicting the impact of environmental changes on fish stocks.

1.1.3: BACTERIOPLANKTON

Life in the ocean is dominated by microbes. Earth's ocean is estimated to contain 10^{29} bacteria (Whitman *et al.*, 1998). It is estimated that 0.1-1.0 ($x10^6$) cells/ml of bacteria and 1-4 ($x10^6$) cells/ml of virus are present in water. Viruses, including bacteriophages, are also important to control the bacterial population as they attack and kill bacteria, archaea and other microorganisms, which releases the dead cells content, adding to the organic matter in the water (Zimmer, 2006). These small single-celled organisms constitute the base of the marine food web and catalyze the transformation of energy and matter in the sea.

Recent discoveries have shown that small plankton-bacteria and micro-grazers (microzooplankton) are key to maintaining the flux of carbon and energy within the marine ecosystem thus important in earth's carbon and nitrogen cycles. The solar energy fixed by phytoplankton photosynthesis is channeled to higher trophic levels via two routes. One is the "grazer chain", which is the route from micro-size phytoplankton to mesozooplankton (e.g. Riley 1947). The other is the "microbial food web", which includes the "microbial loop" consisting of heterotrophic bacteria, protozoans (Azam *et al.*, 1983) and all pro- and eukaryotic unicellular phytoplankton such as pico (0.2–2mm), nano- (2–10mm) and micro-size phytoplankton (Sherr and Sherr, 1988). The final link in all the food chain is made up of decomposers, those heterotrophic bacteria that breakdown dead organic matter and release nutrients back into the marine ecosystem. The dissolved organic carbon (DOC) produced by algae (exudation) and during protozoan and zooplankton grazing is converted into particulate biomass by bacteria and thereby re-channeled into the marine food web (Azam *et al.*, 1983 and 1998; Gauns, 2000). Most of the carbon in the marine ecosystem is cycled by microorganism through "microbial loop" (Azam *et al.*, 1983) (Fig. 1.2).Thus, microbial loop strongly influences the quality, quantity and size distribution of food available to higher organisms.

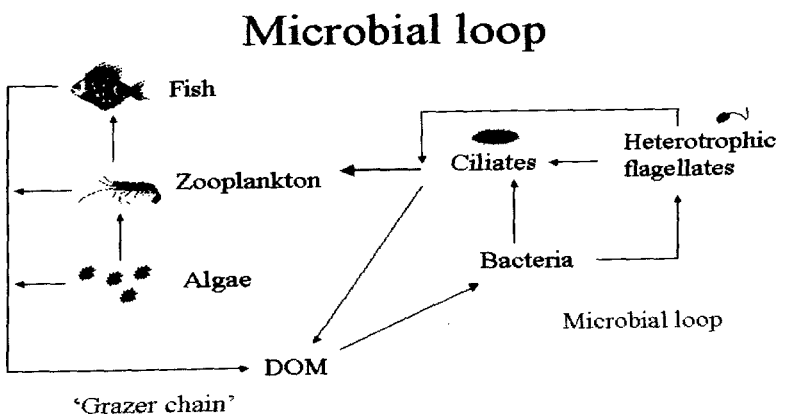


Fig. 1.2. Microbial loop, explains the role of microbes in transformation of energy in marine ecosystem.

Many studies have been done on the planktonic food web in offshore waters around the world, such as in the NE subarctic Pacific (Booth *et al.*, 1993, Boyd *et al.*, 1995a and b; Yamaguchi *et al.*, 2002), western North Atlantic (Harrison *et al.*, 1993), Sargasso Sea off Bermuda (Caron *et al.*, 1995, Roman *et al.*, 1995), Mediterranean Sea (Siokou-Frangou *et al.*, 2002) and the equator at 175°E (Ishizaka *et al.*, 1997). Studies carried out in the Arabian Sea; suggest the importance of microbial loop which operates along with the classical food chain during low productive season (Gauns, 2000). In highly productive regions such as upwelling areas and some temperate waters, the grazing food chain might be the dominant route (Cushing, 1989). Although the grazing food chain is thought to efficiently transfer organic carbon from low to high trophic levels (Cushing, 1989), the microbial food web contributes less to high trophic levels since there are many trophic levels with the associated inevitable higher metabolic costs at each level (Roman *et al.*, 1995; Rousseau *et al.*, 2000). In the water off Cape Esan, the grazing food chain is the predominant route of carbon flow in spring. On the other hand, the microbial food web might be the predominant route in other seasons (Shinada, 2008). Similarly in Japan, studies have also been reported from coastal waters such as the Ariake Sound (Nakamura and Hirata, 2006), Uwa Sea (Nakano *et al.*, 2004), Dokai inlet (Uye *et al.*, 1998), SetoInland Sea (Nakamura *et al.*, 1994, Uye *et al.*, 1996, 1999), IseBay (Uye *et al.*, 2000) and off Usujiri (Shinada *et al.*, 2005). A few studies on the seasonal changes in the planktonic food web have been conducted in offshore waters such as in the Kuroshio and adjacent waters (Nakamachi, 2003) and in the Oyashio water (Shinada *et al.*, 2001).

Large fraction of the biomass and biological activity in the ocean is microscopic, yet we know little about the microbial ecology of the ocean because only one-tenth percent of bacteria have been cultured. Therefore, studies of the plankton food web are important for our better understanding of the biological productivity of any given marine system in terms of its efficiencies and final yields.

1.2: OXYGEN DEPENDENCY OF PLANKTON

Oxygen is an important resource required for the metabolism and sustenance of all life forms in the marine system. But there are few exceptions of course in plankton as well who adapted themselves even in low oxygen conditions. Marine biologists believe that the decline in ocean oxygen levels is a key factor impacting the spawning behavior of cod as well. Cod eggs are found to be very sensitive to oxygen levels and the current level seems to be approaching the range where they cannot live and hatch (Köster *et al.*, 2005).

According to a study reported in 2002 by NASA and the U.S. National Oceanic and Atmospheric Administration scientists, phytoplankton concentrations have declined by as much as 30 percent in northern oceans since the early 1980s (Boyce *et al.*, 2010; http://www.sciencemaster.com/activity/newsletter/sept_news_02.html). If the phytoplankton in the oceans are becoming depleted, and their CO_2-O_2 gas exchanging ability decreases, a rising level of CO_2, and declining level of O_2, will change in the atmosphere. Thus global warming may lead to lowered oxygen content of the world oceans (Keeling and Garcia, 2002), and expansion of OMZs in selected areas. Study by Bopp *et al.* (2002) have found out that atmospheric O_2 concentration is used to estimate the ocean and land sinks of fossil fuel CO_2, by making use of model results and observations of oceanic O_2 fluxes.

The primary sources of dissolved oxygen are the atmosphere and the process of photosynthesis. Oxygen-using processes, both biological and chemical, counter balance these sources of oxygen. Oxygen is depleted during organism respiration and by decomposition of organic matter by microorganism. The concentration of dissolved oxygen found in a water body and available to the organisms, insects, fish, etc., is the result of many dynamic processes. The key factors influencing dissolved oxygen levels include: excess nutrients, phytoplankton growth, death and decomposition, freshwater and saltwater inflow. Dissolved oxygen concentration is an indicator of water quality and the activity level of the plants and animals living. When the oxygen content of water is under saturated (less than that at equilibrium with atmospheric oxygen), it indicates that organic matter is consumed by organisms faster than it is produced by

the plants. Conversely, when the oxygen concentration is greater than saturation, oxygen is being produced by plant photosynthesis (mostly phytoplankton) faster than it is consumed by all the other organisms. Hence, the oxygen concentration is an index of the balance between processes of food production and food consumption. This balance is a key descriptor of the changing status of the ecosystem. When the balance is disrupted, the oxygen concentration can fall to low levels. The combination of strong bottom water oxygen gradients and high organic matter input to the sea floor creates a stressful but food-rich environment for benthic fauna able to tolerate the severe oxygen depletion (Diaz and Rosenberg, 1995; Levin, 2003). Therefore OMZs are inhospitable to many species, they serve as biogeographic barriers, limiting cross-slope movements of populations (White, 1987; Etter *et al.*, 1999; Rogers, 2000; Weeks *et al.*, 2002). OMZ expansion or shrinkage may promote the evolution of species and genetic diversity maxima at mid-slope depths (Jacobs and Lindberg, 1998; Etter *et al.*, 1999; Ulloa *et al.*, 2001). The extent and severity of OMZs will change with alteration of ocean circulation, temperature and productivity (Reichart *et al.*, 1998; Keeling and Garcia, 2002). When the OMZ moves up the shelf during the southwest monsoon in the Indian Ocean (Banse, 1984) there is a notable drop in catches of fishes and prawns (Sankaranarayanan and Qasim, 1968). Expansion of hypoxia off Namibia, sometimes associated with hydrogen sulfide gas release (Weeks *et al.*, 2002), causes redistribution of biota with negative consequences for fish such as hake, shellfish (e.g. lobsters).Without oxygen at the bottom of the water body, anaerobic bacteria (those that live without oxygen) produce acids. These acids not only increase acidity, but also cause a massive release of phosphorus and nitrogen - two major fertilizers from the organic sediment and into the water column. The same anaerobic bacteria put toxic gases in the water including hydrogen sulfide which is toxic to fish, beneficial bacteria and crustaceans.

Large areas of the bathyl seafloor within oxygen minimum zones and some fjords and basins permanently experience severe hypoxia (<0.5 ml O_2 L^{-1}). Macrofaunal communities within these regions exhibit reduced (or enhanced) densities, low species richness and evenness, and high dominance by annelids. Dominant species exhibit varying lifestyles and nutritional modes. These assemblages differ from shallower communities exposed to seasonal or episodic hypoxia in having: (a) much lower oxygen tolerance thresholds, (b) morphological adaptations to maximize respiratory surface, (c) specialist rather than opportunistic lifestyles and (d) potential

to utilize chemosynthesis-based nutritional pathways. Similarities between the systems include reduced macrofaunal diversity, commonality of family level taxa such as spionid polychaetes, tubificid oligochaetes and ampeliscid amphipods. Temporal and spatial stability of dissolved oxygen concentration appears to be a primary factor regulating community structure and function in both OMZs and shallow coastal hypoxic areas.

1.3: SIGNIFICANCE OF THE STUDY AREA

The Arabian Sea is an ideal region for studying monsoon driven tropical ocean dynamics. It is strongly influenced by semiannual monsoonal reversal winds, which drives the physical processes such as coastal and open ocean upwelling during summer and the surface cooling during winter (Ryther *et al.*, 1966; Smith, 2001).The strength of the monsoon winds is regulated by a thermal gradient that develops from differential heating of land and sea. In summer, (southwest monsoon, June-September), heating of the Eurasian land mass results in low pressure over Asia, while high pressure prevails over the Indian Ocean. The direction of the monsoon winds is then southwesterly. In winter, (northeast monsoon, November-February), cooling of the northern hemispheric land mass results in high pressure over land and low pressure over the Indian Ocean, causing a reversal in the direction of the monsoon winds from southwesterly to northeasterly. This surface circulation of the currents in the Indian Ocean changes, in response to the reversal of monsoon winds and the greatest seasonal variability observed in any ocean basin.

The Arabian basin due to these biannual reversals of winds makes it one of the most productive regions in the world ocean (Ryther *et al.*, 1966; Karl, 1987; Gardner *et al.*, 1999). Recent findings show that open water sustains high primary production (ca.>1.5gm C m^{-2} d^{-1}). During summer, the strong southwest monsoon causes intense upwelling and lateral advection (Bauer *et al.*, 1991; Prasannakumar *et al.*, 2001; Smith 2001; Barber *et al.* 2001; Wiggert *et al.*, 2005) while in winter surface cooling in the north results in enhanced vertical mixing (Krey and Babenerd, 1976; Banse and McClain, 1986; Brock *et al.*, 1991; Banse, 1994; Madhupratap *et al.*, 1996). In both the above cases the photic zone gets nutrients from below which results in high

biological productivity (Madhupratap *et al.*, 1996). Studies on primary productivity carried out shows highest during SWM (950 mg C m^{-2} d^{-1}),intermediate during NEM (510 mg C m^{-2} d^{-1}) and lowest during SPIM (210 mg C m^{-2} d^{-1}) (Gauns *et al.*, 2005). High surface productivity in the above two seasons leads to considerable flux of organic particles to deep water (Nair *et al.*, 1989; Hakke *et al.*, 1993; Ramaswamy and Nair, 1994; Rixen *et al.*, 1996) and high rates of oxygen consumption.

The Arabian Sea also has a global significance as it is one of the world's most intense oxygen deficient zone with O_2< 0.1ml/l (Sewell and Fage 1948; Wyrtki 1962, 1973; Qasim 1982; Swallow 1984; Naqvi 1987; Kamykowski and Zentara 1990; Olson et al 1993; Morrison *et al.*, 1999). The increased biological production leads to the formation of "oxygen minimum zone" and can extend up to hundreds of meters vertically and thousands of kilometers horizontally. The core of OMZ occurs at about 150-500m depth in the Arabian Sea and could extend to 1000m depth and in the Bay of Bengal a thinner layer (200-600m) (Naqvi *et al.*, 2006a). Of the total OMZ area, approximately 31% occurs in the eastern Pacific Ocean, 59% in the Indian Ocean (Arabian Sea and Bay of Bengal) and 10% in the southeastern Atlantic (Fig. 3). Lately, two additional seasonal OMZs at high altitude have also been indentified: the West Bearing Sea and the Gulf of Alaska (Paulmier and Ruiz-Pino, 2009).

Vinogradov and Voronina (1961). Dissolved oxygen being an important resource for efficient metabolism (Eckerts, 1983; Hochachka and Somero, 1994) influences the distribution and diel migration of zooplankton taxa below the thermocline. Persistence of pronounced OMZ, the distribution of zooplankton decreases in biomass with depth (Angel, 1990). Previous studies has shown that in the Arabian Sea and Southern end of California current plankton biomass are high in MLD (mixed layer depth) and decreases sharply when oxygen concentration is <0.2ml l^{-1} (Vinogradov and Voronina, 1962; Longhurst, 1967; Bolter-Schnak 1996).

Nevertheless, oceanic animals have modified metabolic system for surviving in OMZ (Childress and Thusen, 1992). Childress and Siebel (1998) proposed 3 general approaches that OMZ taxa can use to cope with low oxygen: (1) increased effectiveness of oxygen uptake (2) lower metabolic demands, and (3) use of anaerobic metabolism. Seafloor OMZs are regions of low biodiversity and are inhospitable to most commercially valuable marine resources, but support a fascinating array of protozoan and metazoan adaptations to hypoxic conditions (Helly and Levin, 2004). Most crustaceans have specialized adaptation to increase their efficiency of removing oxygen from water (Childerss and Seibel, 1998) through their large gill surface, short diffusion distance and respiratory proteins with high oxygen affinity. An organism like the Vampire Squid, a cephalopod, possesses specific physiological adaptations to survive by extracting oxygen from the water more efficiently (Seibel *et al.*, 1999). One strategy used by some classes of bacteria in the oxygen minimum zones is to use nitrate rather than oxygen (Froelich *et al.*, 1979; Lam and Kuypers, 2011)

Some vertical migrators including Copepod *Gaussia princeps* and some fishes may use anaerobic metabolism pathway while temporary in OMZ (Childress, 1977). However, it is remarkable that few species of Copepod and Euphausids make up the whole population of zooplankton present in the low oxygen layer (Vinogradov and Vironima, 1961; Haq *et al.*, 1973; Brinton, 1979). Copepods like *Pleuromamma indica* can migrate in and out of the OMZ of the Arabian Sea where oxygen can be as low as 0.1ml/l (Saraswathy and Iyer, 1986; Smith 1982). *Lucicutia grandis* is yet another species found at 600 -1000m depth and is a good indicator for the lower OMZ interface of the Arabian Sea and Eastern Tropical Pacific (Gowing and Wishner, 1992, 1998; Wishner *et al.*, 1995; Saltzman and Wishner, 1997 b; Morrison *et al.*, 1999). Other

forms such as *Calanoides carinatus* and *Eucalanus subtenuis* dominate surface waters during upwelling, build up lipid reserves in the body and diapauses at deeper depth during non-upwelling period (Smith *et al.*, 1998, 2001). Former species in particular is yet not being reported along the west coast of India. Ostracods are commonly found in the west coast of India and are reported to exist below the thermocline (Padmavati and Goswami, 1996; Madhupratap *et al.*, 2001).

There have been more recent attempts to characterize the vertical zonation of zooplankton of the northern Arabian Sea (Smith, 1982; Madhupratap and Haridas, 1990; Madhupratap *et al.*, 1990; Paulinose *et al.*, 1992; Böttger-Schnack, 1994, 1996; Madhupratap *et al.*, 1996b; Padmavati *et al.*, 1998; Wishner *et al.*, 1998; Smith and Madhupratap, 2005; Wishner *et al.*, 2008). Several studies have been focused on zooplankton biomass during upwelling in the summer and in the fall intermonsoon in the western Arabian Sea (Smith *et al.*, 1998; Stelfox *et al.*, 1999; Smith and Madhupratap, 2005). A few species belonging to the families Metridinidae and Augaptilidae were characteristics of low oxygen examined during the Fall Intermonsoon (Madhupratap *et al.*, 2001). Many of these authors also noticed the absence of pronounced diel variations with respect to surface plankton in the area, which may indicate the inhibition due to the OMZ.

Studies from the West coast of India (Padmavati and Goswami, 1996a, 1996b; Padmavati *et al.*, 1997; Achuthankutty *et al.*, 1998; Goswami *et al.*, 2000) indicates that the distribution of zooplankton taxa were influenced by season, depth of the sampling station and prevailing hydrographic conditions. Zooplankton composition and abundance studied in response to upwelling has shown high biomass confined to narrow coastal belt in the upper shallow mixed layer where few species of copepods like *Temora turbinate* and *Acrocalanus* spp were dominant and distinct from non-upwelling and offshore waters (Madhupratap *et al.*, 1990). Temporal and ephemeral work has been done to study variation in copepod community in the Mandovi-Zuari estuary (Dalal and Goswami, 2001). Tidal variation in zooplankton shows that, low salinity favours high biomass in Zuari estuary (Goswami *et al.*, 1979).

As said earlier, Arabian Sea productivity is regulated mainly by nutrient inputs from below the euphotic zone via upwelling and convective mixing. Several studies have also been carried out on temporal and spatial variation of primary productivity and phytoplankton biomass in the Arabian Sea (Qasim, 1977; Banse, 1987; Bhattathiri *et al.*, 1996; Gauns *et al.*, 2005). Study on changes on biological and physico-chemical parameters were examined during various stages of upwelling during SWM in the south-eastern Arabian Sea (Haeebrehman *et al.*, 2008), found that phytoplankton were dominated by *Nitzschia seriata* and *Rhizosolenia alata* (pennate diatoms). Similarly Smith and Codispoti (1980) reported *Nitzschia delicatissma* and *Rhizosolenia styliformis* were characteristics species in the upwelling region off Somalia.

1.5 OBJECTIVES OF THE STUDY

In addition to the perennial open ocean OMZ, oxygen-deficient conditions (hypoxia-suboxia-anoxia) also develop seasonally (during late summer and autumn) over the continental shelf of India leading to denitrification (Naqvi *et al.*, 2000). Zooplankton composition and abundance have been observed to respond to coastal upwelling with high biomass, however, these studies have been based on very limited spatial and temporal coverage. Studies on other plankton (phytoplankton and picoplankton) and other biological parameters in relation to the seasonal changes in oxygen levels and extremely steep spatial gradients of oxygen along the west coast of India are yet to be undertaken in a systematic manner.

This piece of research work focuses response of biological parameters, to this unique ecosystem. Present study was carried out at already existing long-term monitoring programme called the Candolim Time Series (CaTS) site located in Goan coastal waters. The CaTS site forms a part of a coastal transect comprising five stations G1-G5, visited on a monthly basis using a small vessel for a period of 2 years excluding the peak monsoon months. Likewise, Zuari estuary which is strongly influenced by coastal waters is also considered as a part of the West coast to carry out experimental studies. While, open oceans cruises were conducted in the Northern/Central Arabian Sea during the late monsoon (upwelling) period. These field measurements were carried out to address following objectives.

The main objective of this work includes

- To determine the spatio-temporal variability in the abundance and composition of phyto- and zooplankton communities along the central west coast of India.
- To understand the physico-chemical environmental processes controlling planktonic abundance and composition with special focus on dissolved oxygen.
- To study species succession of phytoplankton in the coastal ecosystem and their variability with space and time.
- To compare the ecological conditions existing in the coastal and open ocean environments and understand the factors responsible for the observed differences.

CHAPTER 2

STUDY AREA

2.1: INTRODUCTION

Three environments selected for this study namely Zuari estuary, the continental shelf region and the open waters of the northern Arabian Sea are contrasting in many physical and chemical and biological characteristics. The present research focuses on the spatio-temporal studies of plankton (phytoplankton and mesozooplankton) community in response to varying oxygen conditions in the inner continental shelf margin along the west coast of India. However, in coastal environment seldom or very limited study has been documented previously on this subject. A coastal transect designated as CaTS, that is, Candolim time series location is situated off Goa, which was basically for field observation and monitoring. The significance of this region is that it is affected by seasonal upwelling and episodic nutrient enrichment due to anthropogenic activity from the coast. Attempts are therefore made to undertake systematic study covering the annual cycle at the time series station which was monitored over a period of 2 years (2005-06). In addition to field observations, experimental work was also carried out at the mouth of the Zuari estuary, one of the major estuarine systems along the west cost of India connecting the Arabian Sea. One of the experiments was to study the response of algal community to nutrient enrichment and the other was laboratory based studying effect(s) of reduced oxygen concentration on the survival of the representative community of zooplankton (details are given in Chapter 7). Apart from these coastal regions, the plankton was also studied from open waters of the Arabian Sea. The key difference between these two contrasting regions is that the eastern continental margin of the Arabian Sea experiences seasonal oxygen deficiency during SWM while open ocean waters inherits a perennial oxygen minimum zone (OMZ) which houses the perennial OMZ (Naqvi *et al.*, 2006). These disparities could naturally be expected to be reflected in their flora and fauna of the region which is investigated in the present study.

2.2: OPEN OCEAN

The present investigation from the open wasters is based on the data collected onboard RV *Roger Revelle* in 2007. This expedition was undertaken during the later-half of the southwest monsoon (from 23 August to 16 September 2007). Area between Lat:14° S to 23° N and Long: 56° to 73° E in the northern Arabian Sea was covered during this investigation (Fig. 2.1). The cruise track of the expedition is shown in the Fig. 2.1, which indicates that the sampling started from the inner shelf region on the eastern coast of Arabian Sea, at the time-series site (station G5 [15° 31'N, 73° 39'E]; ~15 km off Goa coast) and sailed acrossed into the open waters of the northen Arabian Sea.

2.2.1: GEOGRAPHICAL SETTING

The Arabian Sea is situated in the north-western Indian ocean, located between (Lat: 10°S- 23°N; Long: 35°E -80°E), and covers an area of about 6.2 x 10^6 km^2. It bounded by the African and Asian landmasses in the west and north, the Indian subcontinent and the Maldives in the east and the equator in the south. In addition, on the west it is connected to the adjoining Persian Gulf and the Red Sea. The continental shelf is generally wide (often exceeding 100km), east of Karachi along the Pakistan coast and all along the Indian west coast with the maximum (350 km) occurring off the Gulf of Cambay. Elsewhere, the shelf width rarely exceeds 40 km. The Arabian Sea receives lower volumes of river runoff as very few major rivers (Tapti and Narmada) empty into it unlike the Bay of Bengal. The evaporation far exceeds precipitation and runoff, except off the west coast of India where annual precipitation is slightly in excess (<20 cm) over evaporation (Venkateswaran, 1956). The excessive evaporation results in high surface salinities in the Arabian Sea. The two marginal seas, *viz.*, the Red Sea and the Persian Gulf, lying in arid zones experience still more intense evaporation. Consequently, the surface waters are the least saline in the SWM and most saline in the NEM (Wyrtki, 1971) The Arabian Sea experiences extremes in atmospheric forcing, asymmetrically distributed over the region that bring about exceptionally large hydrographical changes and produce a wide variety of ecosystems or biogeochemical provinces. The diversity and spatio-temporal proximity of these provinces make the region a natural laboratory to investigate present biogeochemical processes,

and to apply this knowledge for reconstructing past changes, as well as for prediction future responses of oceanic ecosystems to human-induced climatic change. It has therefore attracted a great deal of attention of oceanographers world over, and has been subjected to numerous investigations such as the International Indian Ocean Expedition (IIOE), GEOSECS (Geochemical Ocean Sections, led by United States), World Ocean Circulation Experiment (WOCE), Joint Global Ocean Flux Study (JGOFS) and The Global ocean ecosystem dynamics (GLOBEC) have immensely improved our understanding of biogeochemistry of this region.

2.2.2: HYDROGRAPHY AND CIRCULATION

The Arabian Sea experiences extremes in atmospheric forcing that leads to the greatest seasonal variability observed in any ocean basin. Changes in the monsoon winds generate coastal and equatorial Kelvin waves and equatorial Rossby waves, having both annual and sub-annual periods, which propagate rapidly through the region, strongly influencing circulation at sites far away from their origin (Shankar and Shetye, 1997). Monsoons are the seasonally reversing winds which bring rain to the Indian subcontinent and cause upwelling along the continental margins. Associated with seasonal changes in the wind field, the near–surface oceanic circulation also reverses completely every six months. Major surface currents in the Arabian Sea during, the two monsoon seasons are schematically shown in Fig. 2.2a (Schott and McCreary, 2001). The forcing mechanisms are different, upwelling results from wind effects and Ekman pumping and consequent offshore transport whereas winter cooling is due to convective circulation induced by densification of surface waters (Madhupratap et al, 1996a). However, the latter is mainly confined to the northern AS (ca. north of 15°N).

2.2.2 (A): NORTHEAST MONSOON (NEM)

During winter (northeast, December-March) monsoon, the surface current reverses, becomes poleward carrying low salinity equatorial waters along the west coast of India. North of the equator, the flow is from east to west in the form of the NE monsoon current. Beginning in November this flow becomes the most intense in February and subsides by April (Wyrtki, 1973). A branch of the NE monsoon current turns north and flows along the west coast of India,

bringing low salinity surface waters from the Bay of Bengal. The other branch turns south off Somalia, crosses the equator and merges with the South Equatorial Current. An equatorial counter-current and an undercurrent are also found (Wyrtki, 1973). Surface circulation in the Arabian Sea is generally anticlockwise during this period.

The winds are predominantly north/north-easterlies during this season with wind speed about 6ms^{-1} (Prasanna Kumar *et al.*, 2001b). The air temperature, in general, is low (about 22°C) in the north. Although under the influence of these winds the possibility of upwelling along the eastern Arabian Sea in winter can be expected, but the winds are too weak on to induce any appreciable offshore Ekman transport (Madhupratap *et al.*, 1996a). The cool dry continental air over the northern Arabian Sea enhances evaporation leading to surface cooling (Prasanna Kumar and Prasad, 1996). Apart from the cooling, the decrease in solar insolation results in further cooling of surface waters. Thus, the reduced sea surface temperature (SST) and deepened mixed layer depth (MLD) in the northern Arabian Sea during winter leads to sinking of surface water which sets in convective mixing and brings about injection of nutrients into the surface layers from the upper thermocline region. Consequently, the Arabian Sea surface water north of 15°N experience cooling and densification (Fig. 2.2b).

Recent studies during winter cooling in the north show that 2-4μM nitrate is constantly available in the upper water column during the period (Madhupratap *et al.*, 1996a, Prasanna *et al.*, 2000). This leads to enhanced chl *a* and primary productivity in the water column which was 807 mg C m^{-2} d^{-1} (Prasanna kumar and Prasad, 1996).

2.2.2 (B): SOUTHWEST MONSOON (SWM)

The current pattern seen during winter changes completely with the onset of the SW (summer) monsoon. The reversal actually starts in February and is completed by May. A prominent feature of the large-scale surface circulation during the SW monsoon is the Somali Current. The northward flowing Somali Current reaches its greatest strength in July (Schott, 1983). The west India Coastal Current (WICC) flows towards the equator along the coast of India (Shetye, *et al.*, 1990; Muraleedharan and Prasanna Kumar, 1996; Fig. 2.2a) carrying at its

peak about 0.5 Sv (1 Sv= 10^6 m3 s^{-1}) of water in the north and 4 Sv in the south (52). Surface currents are shallow (75m deep). Below this there are signatures of down-welling and a pole ward undercurrent carrying low salinity waters from southwestern Bay of Bengal (Shetye *et al.*, 1990; Madhupratap *et al.*, 1994). The latter also becomes progressively weak towards north.

While, the overall direction of winds over the northern Indian Ocean is from the south west, strong winds blow with a speed exceeding 30 knots especially along a strongly sheared low-level atmospheric jet (the Somali Jet-Findlater, 1971), the axis of which extends from the Somali coast towards the Gulf of Cambay (Gujrat coast) (See Fig. 2.2b). Wind speeds are generally higher during the summer (average 15ms^{-1}) (Prasanna-Kumar *et al.*, 2001a). During June and July the jet splits over the Arabian Sea when its northern branch progresses across the Indian subcontinent and the southern branch moves eastward to the south of India. As a result, cyclonic wind stress curl forms northern side of the monsoon jet that leads to deeper mixed layer. On the other hand, anticyclonic curl forms in the southeast side of the jet that shallows mixed layer (Muraleedharan and Prasanna Kumar, 1996). This divergence stimulates an intense phytoplankton bloom over ~40% of the surface area of the basin during the SW monsoon (Smith and Bottero, 1977; Brock *et al.*, 1991). This asymmetric distribution of mixed layer depths about the wind maximum during the south west monsoon can be shown to arise from a combination of vertical mixing and Ekman pumping (Smith and Bottero, 1977; Swallow, 1984; Bauer *et al.*, 1991). The, nutrient-rich upwelled water promotes high primary production (>1000 mg C m^{-2} d^{-1}) during the SW monsoon (Bhattathiri *et al.*, 1996) thus, making this basin one of the most productive regions in the world's oceans (Rhyther *et al.*, 1966; Rhyther and Menzel, 1965).

2.2.2 (c): INTER-MONSOON (SPRING AND FALL INTER MONSOONS)

It is only during the intermonsoons periods, April to May (spring intermonsoon) and October to November; fall intermonsoon) the Arabian Sea attains characteristics of a typical tropical structure. In these periods, winds south of 17° N during April-May are predominantly northerly and weak (<4 m s^{-1}) but become westerlies and progressively stronger towards the north-west. SST increases to 28-29°C. The core of Arabian Sea High Saline Water (ASHSW) is closer to the surface in the north but deepens to about 80 m in the south (Prasanna Kumar and

Prasad, 1996). A shallow mixed layer of 20 to 40 m (Prasanna-Kumar *et al.*, 2001a, b) prevails over most part of the Arabian Sea leading to strong stratification (Wyrtki, 1973) and levels of nutrients become almost undetectable in the upper layers and making the region oligotrophic owing to very low chl *a* and productivity with <200 mg C m^{-2} d^{-1} (Bhattathiri *et al.*, 1996).

2.2.3: OXYGEN MINIMUM ZONE (OMZ)

The Arabian Sea is one of the world's most intense oxygen deficient zones with 0_2 < 0.1ml/l (Sewell and Fage, 1948, Wyrtki, 1962, 1973; Qasim, 1982; Swallow, 1984; Naqvi, 1987; Kamykowski and Zentara, 1990; Olson *et al.*, 1993; Morrison *et al.*, 1999). The upwelling and winter cooling periods bring more nutrients in the euphotic zone leading to higher phytoplankton biomass and primary production. High rates of primary production, high export production and sluggish subsurface and deep water renewal lead to the formation of "oxygen minimum zone". It can extend for 100's of meter vertically and thousands of kilometers horizontally. The core of OMZ occurs between ~100 and 1200 m depth in the Arabian Sea and in the Bay of Bengal layer is thinner (200-600m). The OMZ in the Indian Ocean is more pronounced in the central northern areas and acute oxygen deficiency is experienced in coastal waters only during summer upwelling (Naqvi et al, 2000, 2006) and in the Bay of Bengal a thinner layer (200-600m) (Fig. 2.3; see Smith and Madhupratap, 2005). Mid water OMZ's are also prominent features in other region of the world ocean including the Eastern Atlantic off NW Africa and in the Eastern Tropical Pacific Ocean (Kamykowski and Zentara, 1990). The distribution of the open-ocean oxygen minimum zones is controlled by the large-scale ocean circulation (Wrytki, 1962).

Various hypotheses have been proposed for the maintenance of the OMZ, including slow advection of water allowing long periods for organic decomposition (Sverdrup *et al.*, 1942) and high local respiration rates due to enhanced production in surface waters (Ryther and Menzel, 1965). The dynamic processes required for the maintenance of the OMZ in the Arabian Sea have been studied recently by Olson *et al.* (1993), who estimated the residence time of water in the OMZ to be ~10 years. An oxygen budget constructed for the OMZ reveals that near-zero oxygen concentrations are maintained by moderate consumption in waters of initially low

oxygen content which passes through the layer at a moderate speed (Olson *et al.*, 1993). Other estimates of the renewal time are lower down to 1 year (Naqvi, 1987; Somasundar and Naqvi, 1988; Naqvi and Shailaja, 1993; Howell *et al.*, 1997). Often OMZs support bacterial denitrification in which nitrate ions are used for oxidation of organic matter; in the process they are reduced to molecular nitrogen with nitrite and nitrous oxide as an intermediate (Codispoti and Christiansen, 1985).

The reducing environment in the OMZ has important biogeochemical consequences for nitrogen and carbon cycling as it is conducive for microbially mediated processes such as denitrification. The only other open oceanic sites that experience mid-depth nitrate reduction are located in the eastern tropical Pacific Ocean off Mexico and Peru (Codispoti *et al.*, 1992). The rate of water column denitrification in the Arabian Sea has been estimated to be 12-33 Tg N y^{-1} (Naqvi and Shailaja, 1993; Mantoura *et al.*, 1993). These results indicate that the Arabian Sea is an area of global significance, accounting for ca. 10-30% of the total oceanic water-column denitrification (Naqvi, 1987; Law and Owens, 1990; Naqvi and Shailaja, 1993).

Furthermore, the upwelling also facilitates the ventilation of greenhouse gases generated in the OMZ, such as CO_2, CH_4 and N_2O to the atmosphere and provides a climatic feedback mechanism. The biogeochemical balance between such processes must be sensitive to the climatic changes. The Arabian Sea OMZ may therefore be a sensitive 'marine barometer' for global climate change (Mantoura *et al.*, 1993).

2.3: THE CONTINENTAL SHELF REGION OFF GOA

2.3.1: GEOGRAPHICAL SETTING OF THE STUDY AREA

The study makes use of an already existing monitoring programme, designed to investigate environmental conditions on a long term basis at a time-series location called CaTS (Candolim Time Series). The coastal site is located in Goan coastal waters at 15° 30' N and 74° 39'E approximately about 15 km off the coast (the village Candolim) Goa coast. The CaTS site forms a part of a coastal transect comprising 5 stations (sta) G1-G5 along the shelf region, visited on a

monthly basis using small vessels (Fig. 2.4a). However, due to logistic reasons (mainly the non-availability of a suitable vessel during the turbulent monsoon season), the sampling has not been as regular as needed for an optimum time series. Nonetheless, sampling was done during the crucial late SWM-early fall intermonsoon (FI) period (August- November). The observations are often extended to the shelf break, when possible, using NIO's coastal research vessel *Sagar Sukti*. Standard hydrographic, chemical and biological measurements are made on all field trips and cruises, and so the environmental data are put together in the right perspective essential for a study like this.

2.3.2: COASTAL UPWELLING AND SEASONAL HYPOXIA

A comprehensive study made by Veradhachari and Sharma, (1967), Banse (1968), Sankaranarayana and Jayakumaran (1972, Sankaranarayana et al, 1978). Shetye (1990), established the occurrence of cold upwelled water propagating from south to north along the coast line along the west coast of India (Fig. 2.4b). These upwelling signatures gradually weakened towards the north (Shetye *et al.*, 1990). McCreary *et al.* (1993) suggested that the most important element of this process is the Rossby wave radiation from the Kelvin waves propagating poleward along the western margin of the Indian subcontinent. Shankar and Shetye (1997) studied the dynamics of Lakshadweep high and low in the Arabian Sea and suggested that as a consequence of the remotely forced Kelvin wave is the formation of a weak, but nonetheless upwelling-favouring coastal current off southwest India. The upwelling records along the eastern boundary show that it is less intense compared to its western counterpart and narrower hugging the coastline and overlying the shelf. It occurs along the southwest coast of India between ca. 8° to 15° N and does not extend to the northern areas. Shetye and Shenoi (1988) studied the annual cycles of wind stress and ship-drift along the coast and showed that local winds could drive the surface circulation. Thus, the forcing mechanisms like upwelling resultant of wind effects and Ekman pumping and consequent offshore transport is one major process influencing the productivity by bringing nutrient rich but low oxygenated waters to the subsurface layer.

Coastal hypoxia is defined as natural and/or anthropogenic dissolved oxygen (DO) depletion in coastal waters to a certain level (e.g. <30% saturation or <2 mg/l= 62.5 μM) (Zhang

et al., 2010). It has become a world-wide phenomenon in the global coastal ocean and causes a deterioration of the structure and function of ecosystems (Zhang *et al.*, 2010). The earliest systematic records of coastal hypoxia appear in literature from Europe and North America in 1910–1920. The shallow continental shelves, hypoxia events induced by upwelling can cause mass mortality of benthic fauna and diminish ecosystem services (Carruthers et al, 1959; Cockroft, 2001; Grantham *et al.*, 2004; Ingole et al, 2010). Thus, hypoxia has a significant effect on benthic animals with the consequences that ecosystem functions related to macrofauna such as bio-irrigation (Rhoads, 1974; McCaffrey *et al.*, 1980) and bioturbation (Aller, 2001; Waldbusser *et al.*, 2004) are significantly affected. Since many microbes and microbial-mediated biogeochemical processes depend on animal-induced transport processes (e.g. re-oxidation of particulate reduced sulfur and denitrification), there are indirect hypoxia effects on biogeochemistry via the benthos. Severe suboxic conditions and at times anoxia leading to production of hydrogen sulphide and consequent mortality of demersal fishes very near to the coast has been reported from the upwelling areas during the southwest monsoon recently (Naqvi *et al.*, 2000). This condition is separate from the oxygen minimum zone (OMZ) which is largely confined to the intermediate layers of the open waters (Naqvi, 1991).

The incidence and extent of coastal hypoxia has risen mainly as a result of increasing human derived discharges of nutrients and organic matter (Diaz and Rosenberg, 2008) causes an increase in re-mineralization of nutrients and certain trace elements (Middelburg and Levin, 2009). An antrhropogenically–enhanced nutrient supply from land or where upwelling brings up subsurface waters of low O_2 content that is further reduced through degradation of copious, locally–produced organic matter. In both cases, a high organic loading is the primary cause of O_2 depletion. This is often associated with strong near-surface stratification, which inhibits vertical mixing and associated aeration of subsurface waters. It is understood that coastal hypoxia has a profound impact on the sustainability of ecosystems, which can be seen, for example, by the change in the food-web structure and system function; or compression and loss of habitat, as well as changes in organism life cycles and reproduction. There are several examples of the anthropogenic type of O_2 deficient environment, popularly known as 'dead zones' because of exclusions of many organisms including commercially important fishes. The largest investigated dead zone along an open ocean is the inner Gulf of Mexico as a consequence of fertilizer runoff

by the Mississippi (Rabalais, *et al.*, 2001). Coastal O_2 depleted environments of the second category are primarily of natural origin due to upwelling, found along the eastern boundaries of the Pacific and the Atlantic Oceans and along the northern boundary of the India Ocean (Helly and Levin, 2004; Kamykowski and Zentara, 1990). Similarly, natural hypoxia has long been known to occur in most coastal upwelling systems, e.g. off Benguela (Copenhagen, 1953; Chapman and Shannon, 1985; Bruchert, *et al.*, 2006), Peru (Dugdale *et al.*, 1977); Gulf of Mexico (Rabalais and Turner, 2001) Of all the naturally- formed O_2 deficient zones, what distinguishes the one over the western Indian continental margin is its pronounced seasonality (Naqvi *et al.*, 2000).

Presently, the study area has been extensively studied on the various biogeochemical changes that occur during later SWM (during late summer and autumn) since 1997. These data reveal well- defined annual cycles of the measured variables, reflecting the seasonality of hydrographic and biogeochemical processes, including the evolution of O_2 deficiency (Fig. 2.4c) Near-bottom O_2 concentrations reach suboxic levels in August as evident by the accumulation of NO_2^- and the depletion of NO_3^- (Fig. 2.4c). Complete loss of the oxidized nitrogen species is followed by the SO_4^{2-} reduction in September–October. With the reversal of surface currents, oxic conditions are re-established in November–December. When the reducing conditions are at their peak in September–October, the cross-shelf sections north of about 12°N latitude (e.g. Fig. 2.4c) show the classical sequence of utilization of electron acceptors: O_2 over and beyond the outer shelf, NO_3^- over the mid-shelf and SO_4^{2-} over the inner shelf. This is perhaps the only known region along an open coast where all three types of redox environments are found on the same shelf segment in such an organized manner. More details of the biogeochemical features of this region are given in Naqvi *et al.* (2006). It has been suggested that, in addition to denitrification, anaerobic ammonium oxidation (e.g. ANNAMOX) is an alternative mechanism regulating nitrogen cycling in the bottom waters of coastal upwelling systems (Kuypers *et al.*, 2003 and 2005; Thamdrup *et al.*, 2006; Devol *et al.* 2006). Complete consumption of nitrate triggers the onset of sulfate reduction (Dugdale *et al.*, 1977; Naqvi *et al.*, 2000, 2006). But, what is lacking is biological response of the community structure (phytoplankton and zooplankton and its composition and abundance) prevailing in such unique ecosystem. Unlike the open-ocean system, the coastal system has intensified over the past few decades presumably due to enhanced

nutrient loading from land (Naqvi *et al.*, 2000, 2006). Further, upwelling systems with oxygen deficient waters can be significant CO_2 sources to the atmosphere in contrast to comparable aerated systems which are typically CO_2 sinks (Santana-Casiano *et al.*, 2009; Naqvi *et al.*, 2009; Taguchi and Fujiwara, 2009).

2.4: ZUARI ESTUARY

2.4.1: INTRODUCTION

The Mandovi-Zuari estuarine system is a well-mixed coastal-plain monsoonal estuary situated between latitudes 15° 25' to 15° 31' N and longitudes 73° 45' to 73' 59' E in Goa, along the west coast of India (Fig. 2.5). They are of major importance and are called the 'lifelines' of Goa. Both rivers have their sources in the Western Ghats. Studies on the Zuari estuary began in the early 1970s and several long and short-term observations have been published from time to time on physical, chemical and biological aspects (Dehadrai, 1970; Cherian et al, 1975; Singbal, 1973; Parulekar *et al.*, 1973; Bhargava and Dwivedi, 1976; Qasim and Sen Gupta, 1981; Qasim, 2003).

Zuari river extends up to ~ 67 km and the width is ~ 5.5 km at the mouth which narrows down to about 0.5 km upstream. Near-mouth region of the Zuari opens into the Arabian Sea. The rivers are the major source of nutrients to the estuaries. Further, region is also subjected to domestic and industrial pollution, which make this environment unique but often vulnerable. They are also sites of high rates of production of organic matter, which not only sustains a secondary food chain internally, but also influence biological productivity of adjacent coastal water in turn sustaining fisheries (Robertson and Alongi, 1992; Wafer *et al.*, 1997). Thus they form the breeding and nursery grounds for several species of important fishes and crustaceans in this region. Thus, Zuari estuary besides supporting a wide variety of animals and plants, act as an important linkage and buffer zone between the ocean and land.

2.4.2: CHARACTERISTICS OF THE STUDY AREA

The physical, chemical and biological features of this estuary are adapted to a seasonal rhythm induced by the annual cycle of the monsoons. It experiences three seasons (1) Southwest monsoon (June-September) when the estuary becomes freshwater-dominated due to heavy precipitation and land runoff. This brings about major changes in temperature, salinity, flow pattern, dissolved oxygen and nutrients. As discussed earlier the peculiarity about the estuarine system is that as it is located along the west coast of India, is under the influence of SWM which drives coastal upwelling. During the upwelling period, low oxygen and nutrient rich cold water from the shelf region penetrates the river mouths (Sankaranarayanan and Jayaraman, 1972). (2) a post-monsoon (October-January) season which is a recovery period from freshwater domination, (3) a pre-monsoon season (February- May) during which the water becomes well mixed and the lower and middle reaches of the estuary becomes marine water dominated. Temperature in the estuary varies between 24.5 and 31.5° C attaining its maximum in April-May and minimum in June – July. Overall, this region generally experiences a humid tropical climate and receives most of the rainfall during the southwest monsoon

The flow in this estuarine system is regulated by the semidiurnal tides; with a maximum range of about 2.3 m. The speed of propagation is about 6.5 ms^{-1} and the tidal amplitude remains unchanged over a distance of about 40 km from the mouth (Shetye, 1999). Oxygen concentration also varies during the tidal cycle, and is also closely related to seasonal changes in temperature and bears an inverse relationship with salinity (Qasim and Sen Gupta, 1981). Salinity also undergoes large variability. During premonsoon, the estuary becomes an extension of the adjacent sea and saline water penetrates upstream region as far as 55 km from the mouth (Qasim and Sen Gupta, 1981).

The estuarine diatoms tolerate a wide range of salinity (2.5-31.5) while dinoflagellates form blooms at salinities between 25.5 and 31.8 (Devassy and Goes, 1988). However, only those organisms that have adapted to wide range of fluctuations can flourish. Therefore, the estuarine environment species are few but are often present in large numbers.

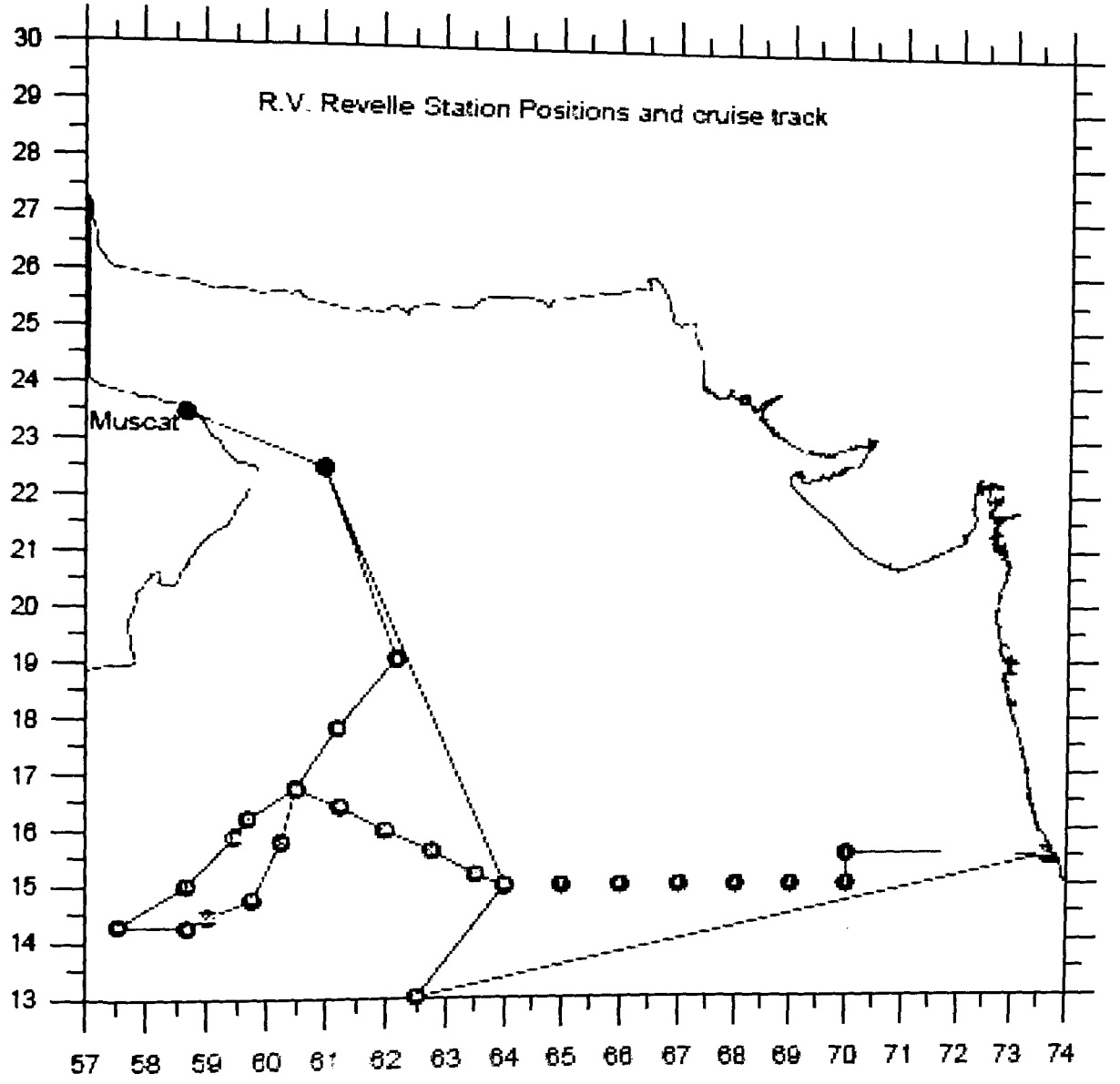


Fig. 2.1: Map of the study area showing cruise track in the Northern Arabian Sea.

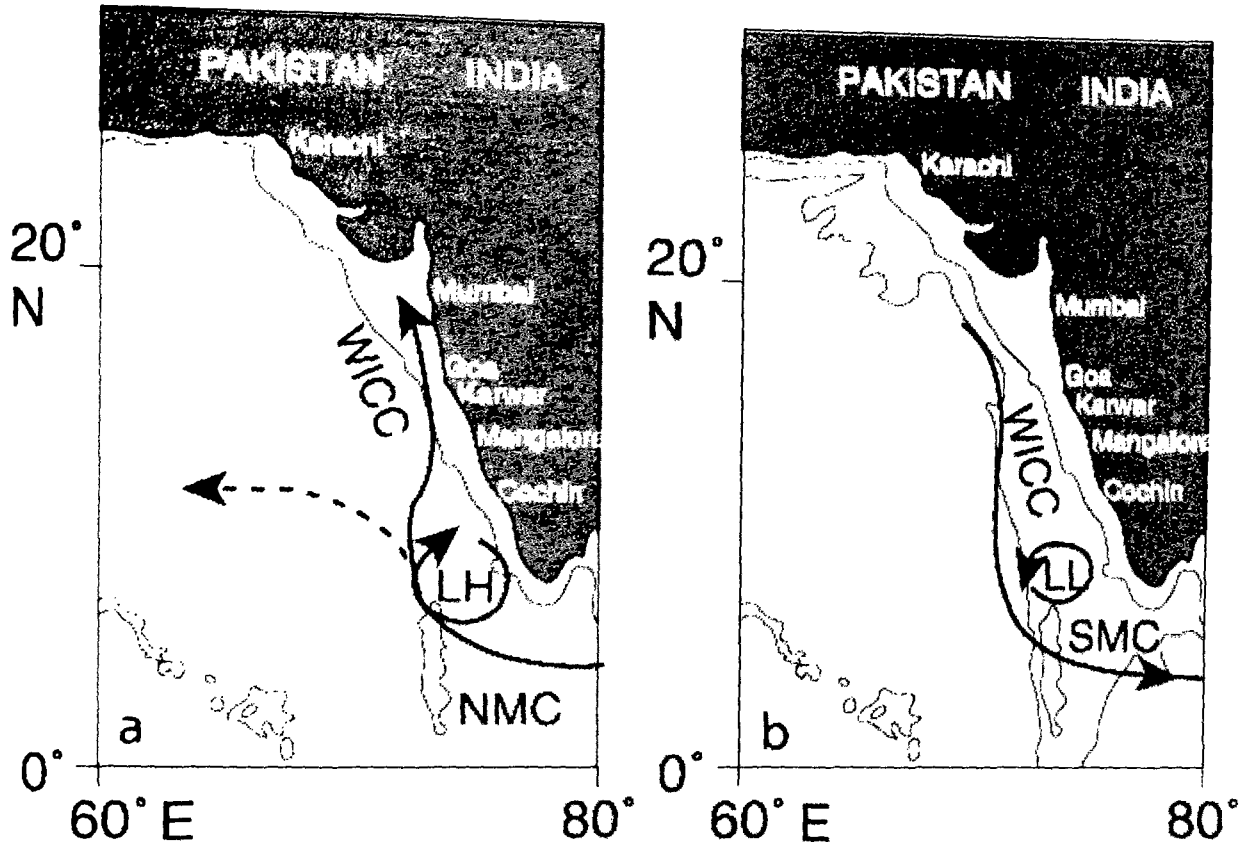


Fig.2.2a: Major features of surface circulation in the eastern Arabian Sea during (a) Northeast Monsoon, and (b) Southwest monsoon (RHJ-Ras-al-Hadd Jet; WICC- West Indian Coastal Current; LH-Lakshsdweep High; LL-Lakshsdweep Low; NMC- Northeast Monsoon Current; SWC- Southwest Monsoon Current (Schott and Mc Creary, 2001)

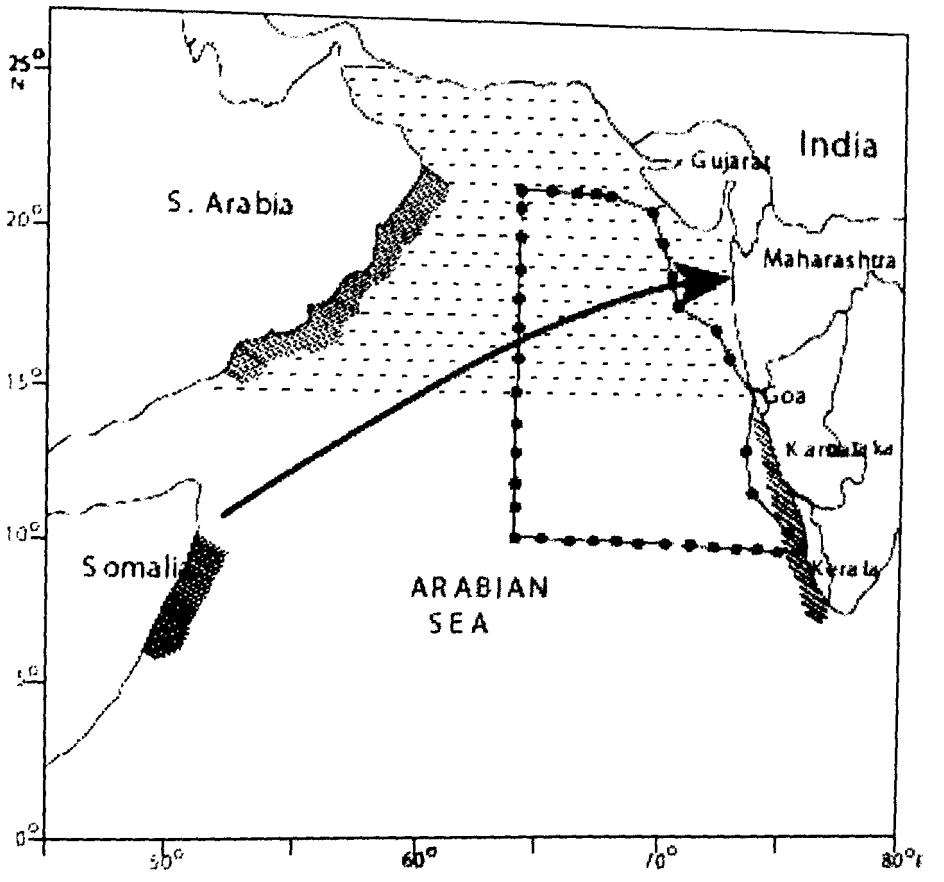


Fig. 2.2b: Diagrammatic representation of Somali jet and upwelling areas. Bold arrow shows the axis of the jet. Hatched areas along Somali, Arabia and the southwest coast of India show regions of coastal upwelling dring SWM and the dotted area in the northern region represents NEM (Madhupratap et al. 2001)

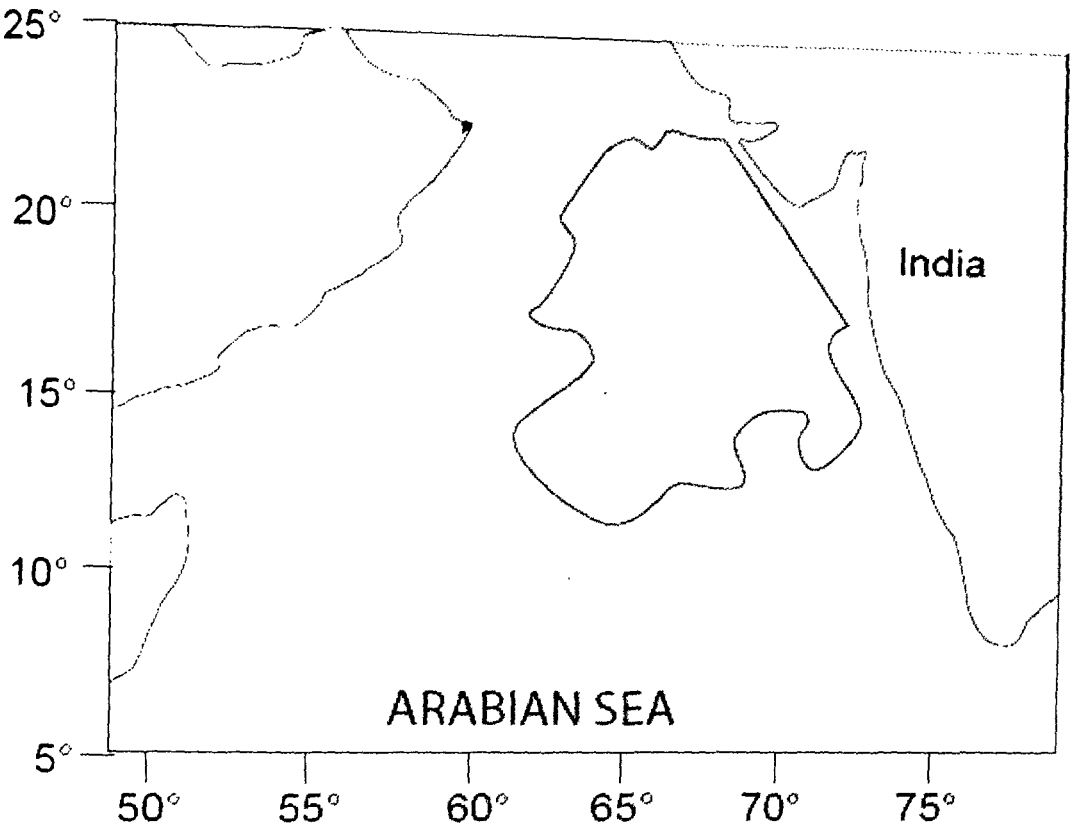


Fig. 2.3: Northern-central Arabian sea in shaded portion depicting the OMZ (redrawn from Smith and Madhupratap, 2005)

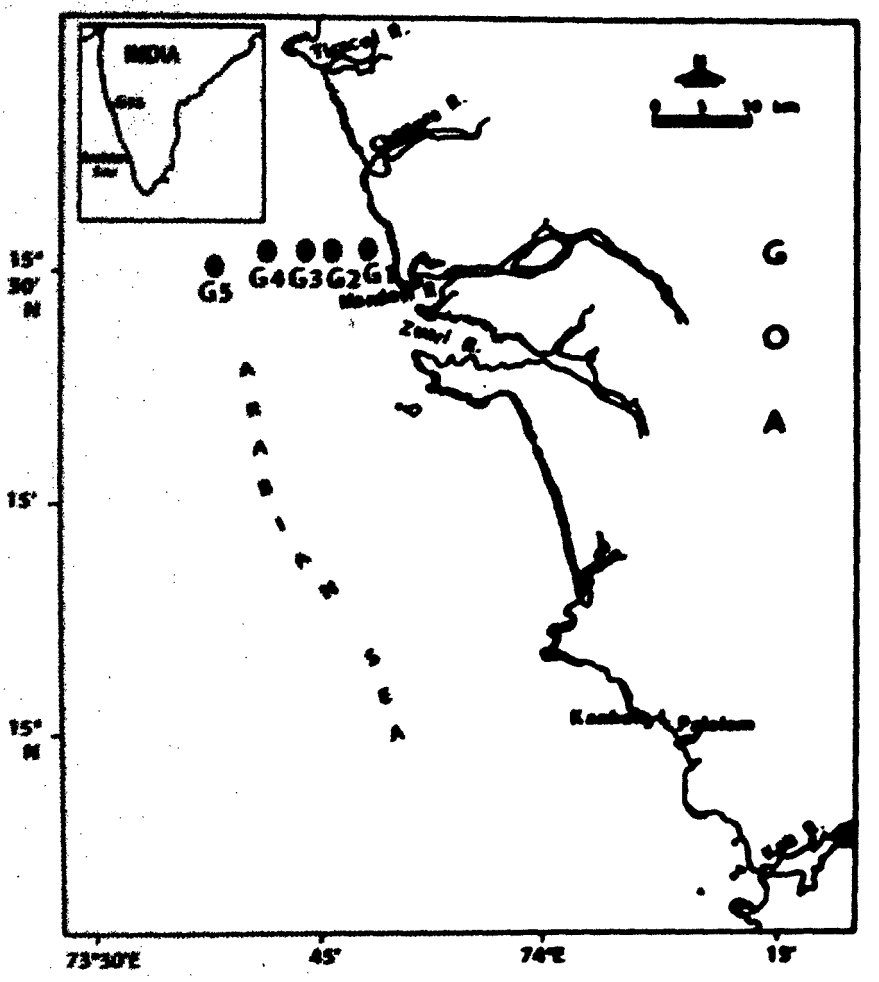


Fig. 2.4a: Map of the study area showing locations at **Candolim Time Series Station (CaTS) G1-** G5 along the west coast of India.

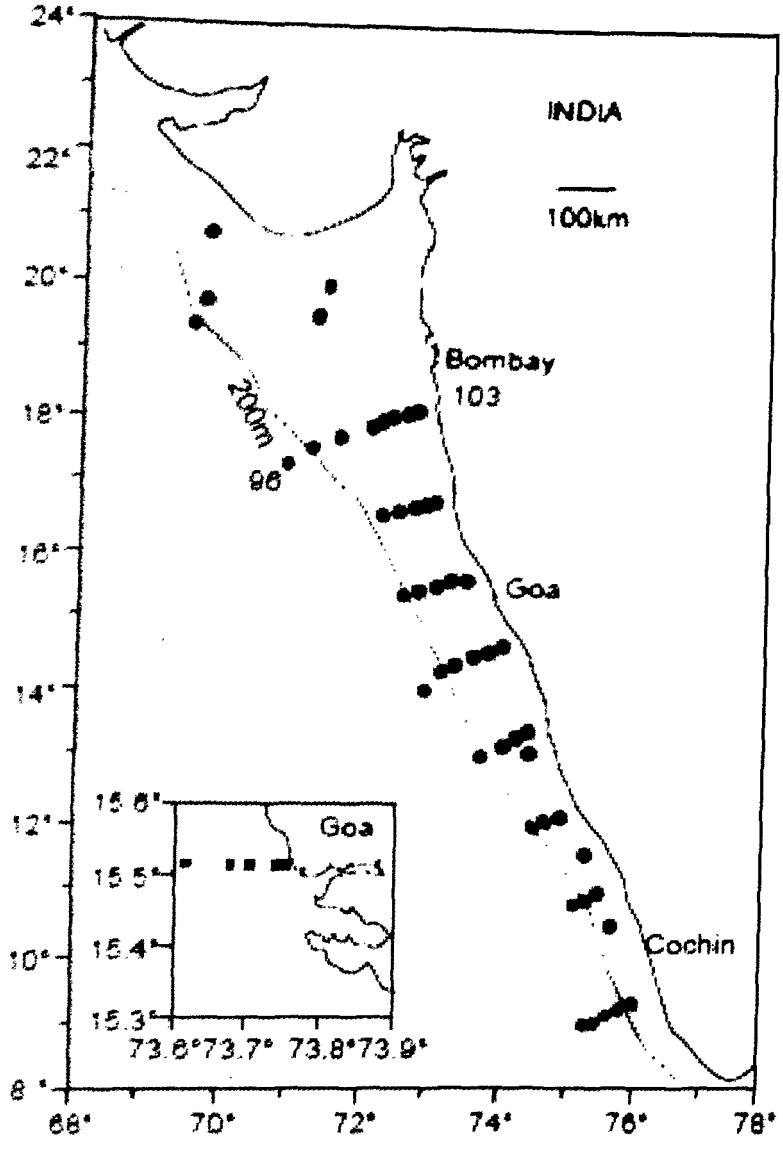


Fig. 2.4b: Map showing the western continental shelf of India, shaded region indicating coastal upwelling during SWM (redrawn from Naqvi et al., 2000).

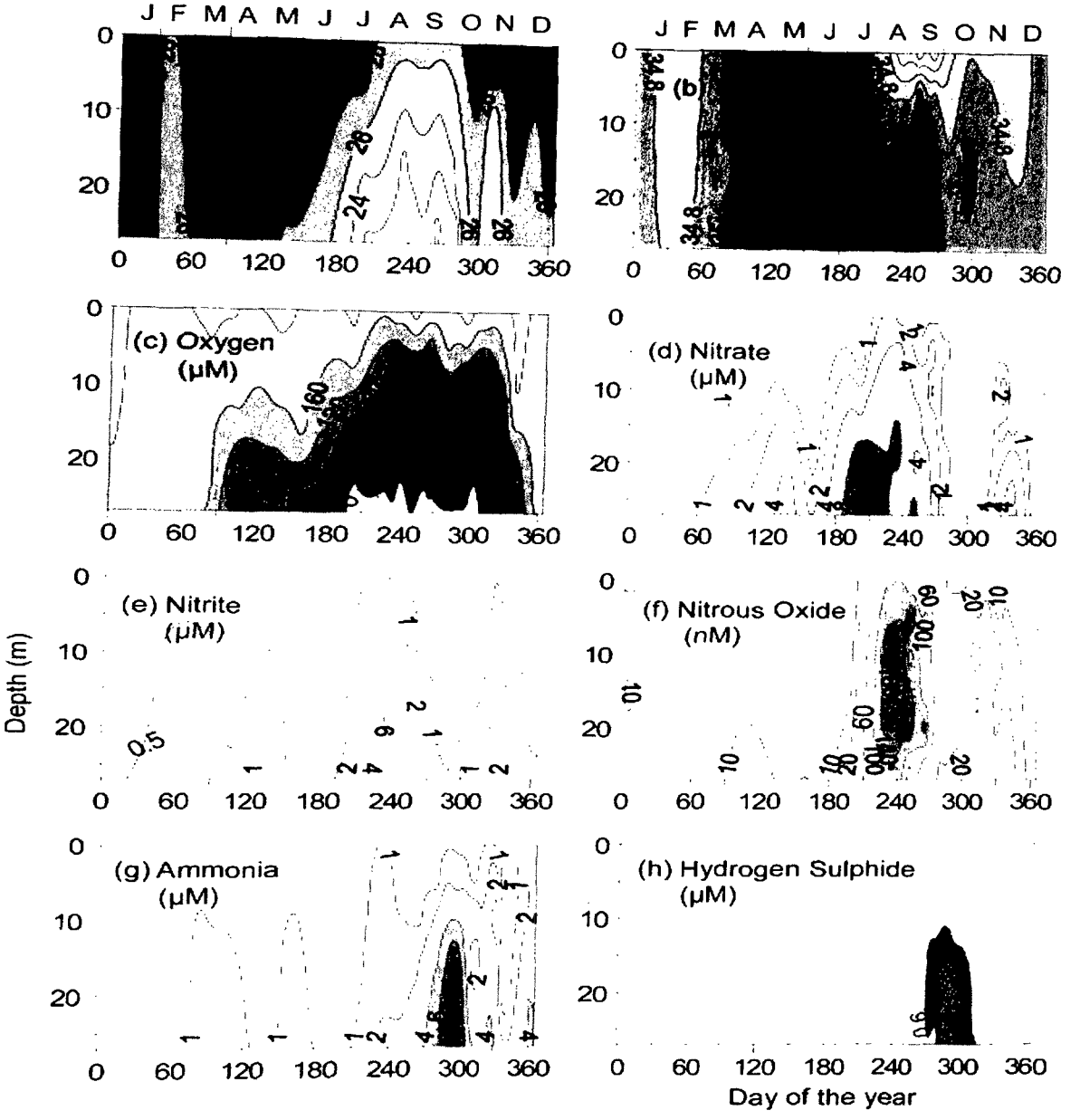


Fig. 2.4c: Monthly/fortnightly averaged records showing annual cycle of (a) temp,(b) salinity, (c) dissolved oxygen,(d-g) inorganic nitrogen species, (h) hydrogen sulphide and (i) chlorophyll *a* at CaTS site based on the observations from 1997-2004 (taken from Naqvi *et al.*, 2006). Coastal upwelling associated with southwest monsoon leads to marked changes in oxygen distribution

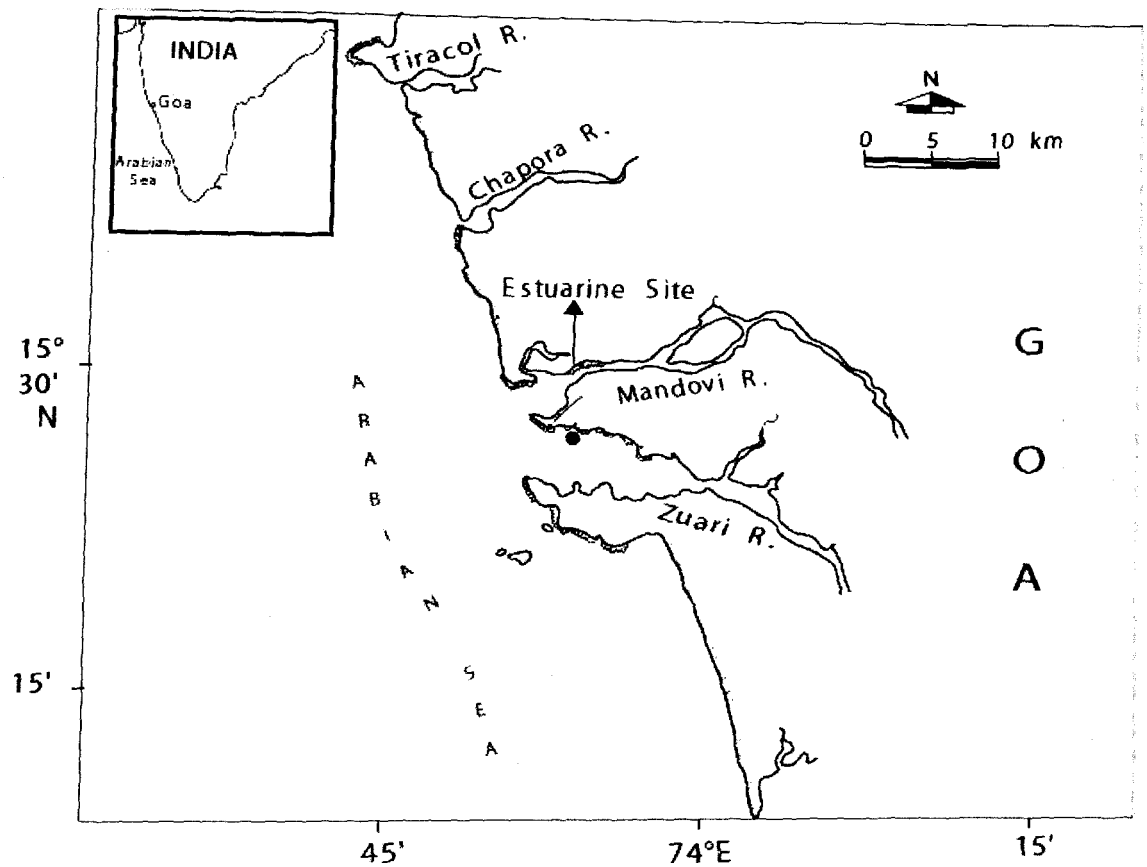


Fig. 2.5: Map showing experimental site in the Zuari estuary.

CHAPTER 3

MATERIALS AND METHODS

In order to meet the objectives of the present study, detailed description of field observations and experimental work, sampling procedures, materials used and analytical/experimental methods adopted or developed are given below.

3.1: FIELD OBSERVATIONS

3.1.1: COASTAL SAMPLING

Various locations of sampling stations from coastal and Open Ocean waters of the Arabian Sea are given in Chapter 2. Field trips were organized on a regular monthly basis to the coastal station from the inner shelf region from five stations *viz.*, G1 to G5 off Goa, using either hired mechanized trawler or NIO's coastal research vessel CRV Sagar Sukti. The samples were collected for a period of two years from December 2004 to December 2006. However, the observations could not be made during early SWM period because of rough seas and also a ban on boat traffic by the captain of Ports, Govt. of Goa. But, towards the later part of the monsoon season i.e. in August/September sampling was resumed. Water samples from selected depths were obtained by 5 L capacity Niskin samplers (General Oceanics, Miami FL, USA) manually while operating on mechanized boats. Samples from Niskin bottles were always first sampled for dissolved gases such as oxygen and then for various other parameters. While sampling for DO, utmost care was taken to avoid any atmospheric contamination through bubbling. Dissolved oxygen were chemically fixed immediately soon after collection. Samples for phytoplankton, mesozooplankton, samples were immediately fixed/ preserved following standard protocol (see below). Primary production measurements were carried out at few stations only restricting to near surface depth. Samples were deck-incubated with ^{14}C-radioactive isotope on board using appropriate light screens. Samples for chlorophyll *a* and nutrient analysis were kept in an ice box soon after collection. Temperature was measured by using reversible thermometer. Salinity and pH samples were collected and stored away from heat/light until analysis. A portable CTD (SBE) profiler was used on board Sagar Sukti for continuous profiling of water column.

3.1.2: OPEN OCEAN SAMPLING

In Open Ocean, water samples from the desired depths were collected by a CTD rosette and used for analysing various biological parameters *viz.* total and size fractionated chlorophyll *a*; phytoplankton; primary productivity and mesozooplankton and chemical parameter (pH, nutrients, dissolved oxygen). Primary productivity was done using PP mooring system incubated *in-situ* conditions covering euphotic zone. Profiles of temperature and salinity at each sampling station were obtained from respective sensors fitted on to a Sea Bird Electronics Seasat (SBE 19, USA) CTD.

3.2: METHODOLOGY/ANALYSIS

BIOLOGICAL PARAMETERS

3.2.1: PHYTOPLANKTON

3.2.1.1: FIELD SAMPLE

For enumeration and speciation of phytoplankton cells (>5μm), 250 ml of sea water was collected from the euphotic zone (0-120 m), while those from shallow coastal depths were as per the standard depth (0, 10, 20, 40, 60, 80, 100 and 120m). Samples were transferred to plastic bottles and fixed with 1% Lugol's iodine and preserved with 3% formaldehyde solution. The advantage of Lugol's iodine solution is that it has an instant effect and increases the weight of the organisms reducing sedimentation time. Lugol's iodine solution may cause discolouration of some phytoplankton making identification difficult. Thus, to reduce this effect, the samples were bleached using sodium thiosulfate prior to analysis. The samples were stored in dark at low temperature (5 °C) to prevent the degradation of Lugol's iodine in light until enumeration which was usually within one month after collection. A settling and siphoning procedure was followed to obtain 20-30 ml concentrate. Two replicates of one ml each of this concentrate was then mounted on a Sedgwick Rafter counting chamber and examined under Olympus inverted microscope (magnification 100-200x). Phytoplankton cells containing pigment content were enumerated and the empty cells were excluded. Generic and species identification was done according to various standard taxonomic keys (Subrahmanyam, 1959; Lebour, 1978; Tomas 1997).

3.2.1.2: EXPERIMENTAL SAMPLE

Water sample in small quantity of 10-50 ml was collected using BD syringes from the experimental bottles. Enumeration and speciation of phytoplankton cells (>5μm) was done following the Utermohl method (Utermohl, 1958). Samples were transferred to plastic bottles and fixed with 1% Lugol's iodine and preserved with 3% formaldehyde solution. Utermöhl (1958) recommended that the bottle to be filled to 75-80% of its volume, which facilitates the homogenisation of the sample before dispensing into the sedimentation chamber. The bottle was shaken firmly, but gently, in irregular jerks to homogenise the contents as violent shaking lead to formations of bubbles, which can be difficult to eliminate. The sedimentation chamber consists of two parts, an upper cylinder (chimney) in volumes of 2, 5, 10, 25 or 50 mL to be used depending upon the algal density and a bottom plate with a thin glass. Sometimes it is necessary to grease the chimney bottom with a small amount of vaseline to ensure the chamber parts are tightly sealed (Andersen and Throndsen 2004). During sedimentation the chambers should be placed on a level, horizontal and solid surface to prevent any non random accumulation of phytoplankton cells. The sedimentation should take place at room temperature and out of direct sunlight. Settling time is dependent on the height of the chamber 10-50 ml sample for setting takes 8-24 hrs. After sedimentation the chamber is gently slide off from the bottom plate and replaced by a cover glass. The bottom plate is placed on the Olympus inverted microscope (100-200X) and the phytoplankton cells are identified and counted.

The transformation of the microscopic counts to the concentration or density of phytoplankton of a desired water volume (usually 1L) can be achieved using this equation:

$$\text{Cells } L^{-1} = N^* (A_t/A_c)^* 1000/V$$

V= volume of counting chamber (ml)

A_t = total area of the counting chamber (mm^2)

A_c= counted area of the counting chamber (mm^2)

N= number of unit (cells) of specific species counted

3.2.2: CHLOROPHYLL (Chl *a*; PHYTOPLANKTON BIOMASS)

By definition the term chlorophyll (chl *a*) denotes a group of photosynthetic pigments important required for carbon fixation and capable of absorbing blue-violet and red light and reflecting blue light. Chlorophyll *a* measurements provide useful estimate of algal biomass and its spatial and temporal variability.

PRINCIPLE OF ANALYSIS

Algal pigments particularly chl *a*, fluoresce in red wavelengths after extraction in acetone when they are excited by blue wavelength of light. The fluorescence was detected on a fluorometer (AU Turner Designs, USA). The fluorometer excites the extracted sample with a broadband blue light and resulting fluorescence in the red detected by a photomultiplier. The significant fluorescence by phaeopigments (phaeo) is corrected by acidifying the sample which converts all of the chl *a* to phaeopigments. By applying a measured conversion for all the relative strength of chl *a* and phaeo fluorescence, the two values can be used to calculate both the chl *a* and phaeo pigment concentration using standard equation given in JGOFS protocol (UNESCO 1994)

3.2.2.1: FOR ESTIMATION OF TOTAL CHLOROPHYLL (Chl *a*)

One liter volume of seawater sample is collected in replicates from respective depth and filtered through a Whatman GF/F paper (47 mm diameter, 0.7 m pore size) under low vacuum pressure. Filtration is normally carried out in dim/low light conditions. The pigments were extracted in 10 ml 90% acetone in the dark for 24 hrs in the refrigerator (4°C). Samples were brought to room temperature and the fluorescence was measured at 664 nm on a Fluorometer (AU Turner Designs, USA) which was previously calibrated by chlorophyll *a* standards (Sigma). Fluorescence was measured before and after acidification with two drops of 1.2 N HCl. The chlorophyll *a* was calculated from the fluoroscence using the appropriate calibration factor. The average values from each depth were integrated to obtain the column concentration whenever required.

3.2.2.2: FOR SIZE FRACTIONATED CHLOROPHYLL (Chl *a*)

For size fractionate chl a measurement, a 3L of water sample was passed serially through bolting silk mesh of different pore size filters (200, 60, 20 and 10 μm) under gravitational force. Then, only 1L filtrate was collected from the above filtration and passed through 0.7μm GF/F paper, under low vacuum pressure. Algal biomass retained onto the respective pore size filters was then extracted and analyzed using the protocol described above for total chlorophyll *a* measurements.

The concentrations of chl *a* and phaeo-pigments in the samples were calculated using the following equations:

Chlorophyll *a*

$$\text{Chl } a\ (\mu g/l) = [(F_m/(F_m - 1)] * (F_o - Fa) * (K_x / V_o) * [Vol_{ex} / Vol_s]$$

Phaeopigments

$$\text{Phaeo (Chl equiv. } \mu g/l) = [F_m/(F_m\text{-}1)] * \{(F_m * Fa) - F_o\} * K_x - Vol_{ex}$$

Where
F_m = acidification coefficient (F_o/F_a) for pure Chl *a*
F_o = Reading before acidification
F_a = Reading after acidification
K_x = Door factor from calibration calculations (for 10 ml standard)
Vol_{ex} = extraction volume (ml)
Vol_s = sample volume (ml)
V_o = Volume used for calibration in ml (usually 10 ml)

The instrument (Fluorometer) - was calibrated with Chl *a* standard (Sigma, UK) before and after every cruise by dissolving the standard in 90% acetone for at least 2 hours before it's concentrations (mg L^{-1}) was measured fluorometrically.

3.2.3: PRIMARY PRODUCTION

PRINCIPLE OF ANALYSIS

Primary production is defined as the uptake of inorganic carbon by autotrophic communities for formation of new cellular component in the eventual production of particulate organic matter. The rate of carbon fixation by autotrophs in seawater was measured by tracing the uptake of radioactive ^{14}C ($Na_2 H_{14}CO_3$). Primary production is expressed as "mg carbon m^{-3} d^{-1}". A known concentration of 185 kbq radiocarbon was added to the seawater sample and the uptake of radiocarbon by the phytoplankton was converted to total carbon uptake by using a conversion ratio of added radiocarbon to total inorganic carbon in the sample. Vertical profiles of production measurements were integrated to yield a production rate per unit area in units of mg carbon m^{-2} d^{-1}.

Prior to measuring primary productivity, preparation of sample bottle for PP is important to avoid any external contamination of bottles. Polycarbonate (PC, Nalgene, USA) 300ml bottles used for primary productivity measurements, were soaked for 72 hours in a 5% solution of detergent. These bottles were then rinsed thoroughly with deionized water and subsequently soaked for 72 hours in the acid cleaning solution (0.5N HCl solution prepared with distilled water). Bottles were then rinsed 3 times with Milli-Q water or distilled water. Water samples were obtained from 8 predetermined depths (0, 10, 20,40,60,80,100 and 120m) covering entire euphotic zone and processed for phytoplankton and chlorophyll analyses. From each depth water samples were transferred to five polycarbonate bottles. Before addition of radioactive carbonate, none of the samples were exposed to light (as either light can enhance productivity or degrade/reduce the cell capacity to produce due to light shock in samples particularly from deeper depths). To each polycarbonate bottle containing sea water sample (300 ml) 1 ml of aqueous solution of 185 kbq of radioactive carbon (BRIT, Bhabha Atomic Research Centre, Department of Atomic Energy, and Mumbai) was added. From one bottle, 100 ml sample was filtered on to 25 mm GF/F filter paper for determining the initial adsorption of the ^{14}C by the particles in the sample. From the remaining four bottles from each depth, one was covered with aluminum foil and transferred to a black bag with a velcro closure to determine the dark production. Thus, one dark and three light bottles were used from each depth for *in situ*

incubation for 12 hours from sunrise to sunset. The bottles were deployed *in-situ* to at their respective depths using polypropylene line attached to a buoy. The "mooring" system was deployed approximately one hour before sunrise, and was allowed to drift freely for 12 hours. The system was then retrieved ~ 30 minutes after sunset.

Upon retrieval 100ml each were filtered from each bottle in replicates on to GF/F filter and the filter paper were transferred into 8 ml scintillation vials. Drop of 0.5 N HCl was added to each vial and capped overnight. All vials were held at room temperature until the radioactivity was counted. Before counting, all vials were uncapped and left open overnight. Five ml of liquid scintillation cocktail was added and the radioactivity was counted on the liquid scintillation system (Packard 2500 TR, USA). The count rates were converted to daily production rates (mg C m^{-3} d^{-1}) which were obtained from the triplicates that generally agreed within ± 10% of covariance and were averaged to obtain mean values for a given depth. Dark bottle production rate was subtracted from the mean rate of light bottle to correct for adsorption. Similarly, to determine the initial activity (T_o: Time zero) in the bottles, 0.2 ml of sample was transferred to a scintillation vial containing 0.2 ml of ethanolamine, which prevents the radio labeled l inorganic CO_2 from escaping to the atmosphere.

The daily production rate at various depths was used to calculate the integrated water column production (mg C m^{-2} d^{-1}). The primary production measurements were not carried out during monsoon due to very rough seas conditions.

Calculation:

Primary Production (mg C m^{-3} day^{-1}) = 1.05 x SDPM x W / S_A x T

Sample Activity (S_A)= V * TDPM / A_{Vol}

Where

DPM = disintegration per minute

S_{DPM}= DPMs in filtered sample

T_{DPM}= Total ^{14}C DPMs (in 0.25 ml)

A_{Vol} = volumn taken to measure sample activity

V= volume of filtered sample (liters)

T= time (days)

1.05 = correction for the lower uptake of ^{14}C compared to ^{12}C

W = dissolved inorganic carbon (DIC) concentration in sample (~25000 mg Cm^{-3})

3.2.4: MESOZOOPLANKTON

Mesozooplankton is a group of heterotrophic organisms that depend on organic matter produced by autotrophic (as well as microheterotrophic) organisms for their nutrition and are an important link in the food chain of the oceans.

From coastal stations, the mesozooplankton samples were collected using a Heron Tranter net (mouth area 0.25 m^2; mesh size 200 μm) equipped with a pre-calibrated flow meter. The net was towed vertically from close to the bottom (~27 m) to surface at all 5 stations. During late southwest monsoon, separate cast was made additionally to capture organism living in upper well mixed layer of the water column (10m to surface). The volume of water filtered was estimated using a flow meter (General Oceanics, USA). Immediately after the retrieval of the net, zooplankton samples collected in the plankton bucket at the cod-end were transferred carefully into a plastic bottle. Samples were immediately fixed in 5% buffered formalin solution. Care was taken to wash net thoroughly after every cast to avoid contamination. Further in the laboratory, biomass was determined by displacement volume method. For which, the sample was passed through 200μm nylon mesh to drain as much water and placed on an absorbent paper to remove excess water. The wet biomass was then transferred to a graduated cylinder with a known volume of water. The volume displaced after transferring the samples collection was noted. Buffered formalin (4% V/V) was used to preserve these samples. Depending on the size of the sample; aliquots of 6–25% were obtained using Folsom plankton splitter. Organisms were examined for microscopic identification and enumeration to the generic/species level with the help of using various taxonomic keys using a stereozoom microscope.

In open sea, the zooplankton samples were obtained using a Multiple Plankton Closing Net (Hydro-Bios, mouth area 0.25 m^2, mesh size 200 μm). Five stratified depths samples were collected from each station: 1000-500m, 500-300m, 300-base of thermocline (BT), BT-top of thermocline (TT) and TT-surface (mixed layer). Immediately zooplankton biomass was measured and then sample was preserved using appropriate preservative for other analysis as mentioned above.

3.3: CHEMICAL/PHYSICAL PARAMETERS

3.3.1: DISSOLVED OXYGEN

Dissolved oxygen (DO) in seawater was determined by the Winkler's method as described in Grasshoff *et al.* (1983). Water samples were carefully collected in a glass bottle (125 ml) without trapping air bubbles. Immediately samples were fixed by adding 1 ml of Winkler A ($MnCl_2$) and 1 ml of Winkler B (alkaline iodide) solution. The oxyhydroxide precipitate formed was acidified by adding 50% H_2SO_4 and titrated against sodium thiosulphate using starch as an indicator. The principle of the method is that oxygen in sea water sample reacts with Mn (II). In the presence of excess iodide, Mn (IV) liberates iodine on acidification. The concentration of oxygen was computed from the amount of thiosulphate used for neutralizing the I_2 liberated. The precision of determination was ± 0.05 ml L^{-1}.

The dissolved oxygen analysed by titration method was calculated with the following relation.

$$DO\ (ml/litre) = 5.6 * N * (S-b_m) * V/(V-1) * (1000/A)$$

Where

N = Normality of the thiosulphate
S = Titre value for sample
b_m = Mean titre value for blank
V = Volume of the sample bottle
A = Volume of sample titrated (50ml)

3.3.2: NUTRIENTS

Nutrients were analyzed within 24 hours of sampling. After removing from deep freezer, the water samples were allowed to attain room temperature and then mixed well. The samples were first analyzed together for NO_3^- +NO_2^-, NO_2^-, PO_4^{3-} and SiO_4^{4-}, and then separately for NH_4^+ using a SKALAR segmented flow Autoanalyzer (Model SFA- version 4.X) with precisions ±0.06, ±0.006, ±0.003, ±0.06, ±0.01 µM, respectively. The automated analyses are essentially based on the respective colorimetric methods described by Grasshoff *et al.,* (1983). The primary

calibration standards were prepared with utmost care and accuracy Grasshoff *et al.*, (1983) and preserved at low temperature (4°C). The standards were compared and found to agree well with CSK nutrient standards (Japan).

3.3.3: TEMPERATURE AND SALINITY

Temperature and salinity profiles from the open sea were taken from the respective sensors on the CTD. These profiles were used for relating the influence of these parameters on various biological parameters. In coastal waters for shallow depth it was measured by using reversible thermometer and salinity by Mohr Knudsen argentometric chlorinity titration method (Grasshoff, 1983). Standard seawater of chlorinity 19.374 x 10^{-3} supplied by the Institute of Oceanographic Sciences, U.K. was used for standardizing silver nitrate solution.

Salinity (S x 10^{-3}) was calculated from chlorinity (Cl) from the relation

$Sx10^{-3} = 1.80655 * Cl\ (x\ 10^{-3})$

$Cl = V + K$
$V = V_c \times F$
$F = N/C_m$

Where

F= Standardisation factor
N=nominal chlorinity of Standard Sea Water on the ampoule
V_c=mean value of the amount of $AgNo_3$ consumed.
K=Correction factor for the calculation of chlorinity

CHAPTER 4

SECTION-4A

SUCCESSION IN MARINE PHYTOPLANKTON AND MESOZOOPLANKTON IN RESPONSE TO OXYGEN DEFICIENCY IN WATERS OF THE WESTERN CONTINENTAL SHELF OF INDIA

4.1: INTRODUCTION

Biological productivity in the Arabian Sea is regulated mainly by nutrient inputs from subsurface waters via upwelling in summer and convective mixing in winter (Banse and Mc Clain, 1986; Madhupratap *et al.*, 1996; Barber *et al.*, 2001). The fertilization of euphotic zone leads to high biological productivity during most parts of the year and widespread phytoplankton blooms during both summer and winter (Naqvi *et al.*, 2003). This, in turn, supports high export flux of organic matter to deep sea (Haake *et al.*, 1993; Ramaswamy and Nair 1994; Rixen *et al.*, 2005), which contributes to the development of one of the thickest and most intense oxygen minimum zones of the World oceans (Naqvi, 1987; Olson *et al.*, 1993; Morrison *et al.*, 1999).

Marine organisms including zooplankton exposed to low oxygen environments are subjected to severe stress (Diaz and Rosenberg 1995). Previous studies have shown that in the Arabian Sea and southern part of the California current plankton biomass remains high in the mixed layer but decreases sharply where oxygen concentration falls below 0.2 ml l^{-1} (Vinogradov and Voronina, 1962; Longhurst, 1967; Bolter-Schnak, 1996). Nevertheless, some organisms have modified metabolic systems for surviving in the OMZ. For instance, most crustaceans have a specialized adaptation to increase their efficiency of assimilating oxygen from water (Childress, 1971; Childress and Seibel, 1998) through their large gill surface, short

diffusion distance and respiratory proteins with high oxygen affinity. The jelly fish (*Aurelia labiata*) is known to use intragel oxygen as a reservoir to support its metabolic needs when it migrates into low oxygen waters (Thuesen *et al.*, 2005). On the other hand, decapod shrimps *Gennadas sordidus, Sergia filictum, Eupasiphae gilesii* and *Gnathophausia ingens* are known to live almost exclusively within the OMZ (Childress, 1971; Mincks *et al.*, 2000). It is assumed that some vertical migrators like copepod *Gaussia princeps* and fishes use an anaerobic metabolic pathway temporarily in the OMZ (Childress, 1977). At times, copepod *Pleuromamma indica* and Euphausids make up the entire population of zooplankton present in the low oxygen layer (Haq *et al.*, 1973; Brinton, 1979; Smith, 1982; Saraswathy and Iyer, 1986). The ontogenetic migrant *Calanoides carinatus* is known to live in hypoxic waters (Auel and Verheye, 2007) while *Rhincalanus nasutus, Eucalanus attenuatus* and *Lucicutia maxima* spend a portion of their life cycle in the suboxic zone within the OMZ (Vinogradov and Voronina, 1962). Few other species belonging to the families Metridinidae and Augaptilidae are also characteristic of low oxygen (<0.5ml O_2 L^{-1}) open ocean (Saraswathy and Iyer, 1986; Madhupratap *et al.*, 2001).

In contrast to the perennial OMZs found in the open oceanic areas of the northern Indian Ocean, severe oxygen deficiency also occurs during summer upwelling along the western continental shelf of India, covering an area of 180,000 km^2 (Naqvi *et al.*, 2006). The onset of the southwest monsoon (SWM) in June marks the beginning of upwelling along the SW coast of India (Sarma, 1967; Naqvi *et al.*, 2000) which propagates northward and persists at least until October (Banse, 1959, 1984; Unnikrishnan and Antony, 1992). Upwelling brings up subsurface water having low O_2 content over the shelf. The upwelled water is however capped by a thin low-salinity layer, which is formed as a result of intense rainfall in the coastal zone. Degradation of organic matter in the relatively stagnated unpwelled water and in the underlying sediments quickly removes the residual oxygen below the shallow pyncnocline (Naqvi *et al.*, 2006). Oxygen depleted environments are also found along the eastern boundaries of the Pacific and the Atlantic oceans (Helly and Levin, 2004; Granthan *et al.*, 2004). The total areas of the continental shelf margin exposed to bottom waters having dissolved O_2 <0.5 ml L^{-1} and <0.2 ml L^{-1} are estimated to be 1.15 x 10^6 and 0.76 x10^6 km^2, respectively; of these the contribution of the northern Indian ocean alone is roughly 59 and 63%, respectively (Helly and Levin, 2004). The prevalence of oxygen-deficient conditions in coastal upwelling regions, including the eastern

Arabian Sea, leads to depletion of marine life in particularly benthic fauna (Carruthers *et al.*, 1959; Levin *et al.*, 2009).

Most of the studies on mesozooplankton along the continental shelf of India including the study region have focused on spatial and temporal variability (Padmavati and Goswami, 1996a, 1996b; Padmavati *et al.*, 1997; Achuthankutty *et al.*, 1998; Goswami *et al.*, 2000). Likewise, variability of phytoplankton distribution during the onset, peak and late phases of upwelling in the Arabian Sea has been reported previously by Habeebrehman *et al.*, (2008). However, studies of phytoplankton (especially pico-plankton) in relation to seasonal changes in oxygen content of water along the west coast of India are rare. This paper deals with shifts in community structure of mesozooplankton and phytoplankton along the central west coast of India in response to dissolved oxygen changes that occur on a magnitude rarely seen elsewhere along an open coast.

4.2: MATERIALS AND METHODS

4.2.1: STUDY AREA

The study was carried out at the Candolim Time Series (CaTS) site located approximately 11 km off Goa coast at 15° 31'N, 73° 39'E. The CaTS station (G5, sampling depths: 0, 9, 18 and 25 m) forms the offshore end of a coast-perpendicular transect that also includes four other stations G1 (sampling depths: 0 and 4 m), G2 (0 and 6 m), G3 (0, 6 and 12 m) and G 4 (0, 6 and 15 m) and (Fig. 4A.1). These stations were visited on a monthly basis using a mechanized boat. However, no sampling could be carried out during peak SW monsoon (June-July) due to non-availability of a suitable vessel that could withstand the stormy weather. Nevertheless, peak anoxic conditions usually develop toward the end of the SW monsoon, a period well-covered by our observations. During this period, whenever possible, observations were extended well beyond the CaTS section - to the shelf break - using NIO's coastal research vessel *Sagar Sukti*.

As described below, a suite of physico-chemical and biological measurements were made in order to understand the dynamics of phytoplankton and mesozooplankton communities in response to changes in the dissolved oxygen field over the annual cycle. For the laboratory experiments, dominant representative zooplankton groups were collected from the mouth region of the Zuari estuary which is strongly influenced by coastal waters during high tide.

4.2.2: STATISTICAL ANALYSIS

The data were evaluated by multivariate techniques using PRIMER software package (version 5.2.6, PRIMER-E [Clarke and Warwick, 2001]). Biodiversity indices were calculated using Margalef's species richness (d), Shannon– Wiener diversity (H') and Pielou's evenness (J'). Bray–Curtis similarity matrix was produced on the square root transformed abundance data of phytoplankton and zooplankton. Cluster analysis (group average) was conducted using similarity matrix to produce dendograms.

4.3: RESULTS

4.3.1: FIELD STUDY

Climatological data (see Naqvi *et al.*, 2006a) show that the water column in the study region experiences large changes in the oxygen field on a seasonal basis with oxygen deficient conditions prevailing during and just after the SW monsoon, whereas the water column remains well oxygenated during other periods. Depending upon the prevailing DO levels, the water column was designated as oxic (DO>2 ml l^{-1}), hypoxic (<2 ml l^{-1}), suboxic (<0.2 ml l^{-1}) and anoxic (<0.02 ml l^{-1}). Phytoplankton and zooplankton distributions for each of these categories are described below. Variations in physico-chemical and biological parameters are summarized in Table 4A.1.

4.3.1.1: OXIC (DO>2 ML L^{-1})

As stated above, the water column remains generally well oxygenated from December to April. We observed DO concentrations ranging from 6.67 to 3.13 ml l^{-1} (mean ±SD = 4.09±0.97) during this period. The lowest DO was recorded in April 2006 at 19 m at sta. G5 while the highest was measured at surface at sta. G1 in January 2005. The water temperature varied between 26.95 and 29.19 (27.86±0.90), with the highest and the lowest values coming from May 2005 (sta. G1, surf) and December 2005 (sta G5, 25m) surface respectively. The amplitude of salinity change was relatively small, it ranged between 34.98 and 35.79 (35.24 ± 0.35, Table 4.1). Phytoplankton biomass (Chl *a*) ranged from 0.68 to 16.02 mg m^{-3} (4.46 ± 4.46). Size fractionation analysis showed that pico-autotrophs (<5-0.7μm) on average accounted for 51% of the total algal biomass while, nano- (<20-5 μm) and micro- (>20 μm) phytoplankton contributed 6 and 43%, respectively, to the biomass (Fig. 4A.2).

A total of 96 phytoplankton species were found in the study region considering all seasons. Most of them occurred under oxic conditions (66 species belonging to 41 genera, including 47 species of diatoms and 16 species of dinoflagellates). Phytoplankton abundance ranged from 4 x 10^4 to 5.5x10^5 (avg. 1.2x10^5 ± 1.5x10^5) cells l^{-1}. The complete list of species is given in Table 4.2. In general, diatoms remained the predominant group accounting for >95% of all phytoplankton counts at all stations except during a bloom of prasinophytes that occurred at stn. G1 in January 2005. Diatoms, were largely dominated by the centric forms (e.g. *Chaetoceros curvisetus, Chaetoceros* spp., *Leptocylindrus minimus, Skeletonema costatum*) while pennates included *Cocconeis* spp., *Navicula* spp, *Pseudo-nitzschia* spp., *Thalassionema nitzschioides* and *Thalassiosira* spp. Amongst dinoflagellates, *Heterocapsa* spp., *Gyrodinium* spp., *Gymnodinium* spp., *Prorocentrum dentatum, Scrippsiella trochoidea* and *Protoperidinium* spp. were commonly seen. *Dictyocha* spp was the only representative of silicoflagellate in oxic waters. Phytoplankton species diversity index and evenness in oxic condition were expected to be high, but were found to be low (2.77 and 0.66, respectively) as compared to other oxygen conditions (Table 4A.3). This was due to the occurrence of blooms such as prasinophytes (abundance ~2.8x10^5 cells l^{-1}, 52% of the total phytoplankton community) and dinoflagellates (2.5x10^5 cells l^{-1}, 45% of the

total phytoplankton community) such as *Scrippsiella trochoidea, Prorocentrum dentatum, Heterocapsa* sp., *Gyrodinium* spp. and *Gymnodinium* sp.

The abundance and biomass of mesozooplankton varied from 385 to 15061 (4569±7048) org. m^{-3}, and from 3.1 to 267 (107±128) ml 100 m^{-3}, respectively (Table 4A.1). The predominant (87%) group was Copepoda. In all, 72 species belonging to 33 genera (Table 4A.4) with high species diversity (2.77) and richness (5.44) were recorded from oxic waters (Table 4.3B). Copepods present exclusively in oxic waters were *Calanus tenuicornis, Temora turbinata, T. discaudata, Cosmocalanus darwinii, Centropages furcatus, C. calaninus, Acartia centrura, Candacia bradyi, Calanopia eliptica, Labidocera minuta* and *Oithona rigida.* Suspension feeders like Siphonophores, *Sagitta bedoti* and fish larvae were also reported. Overall, the major copepod community (>2% abundance) comprised of carnivorous (*Oithona rigida* and *Oncea* spp.), herbivores (*Paracalanus* spp., *Canthocalaus pauper)* and omnivores (*Acartia spinicauda, Acartia negligens* and *Acartia centrura)* forms. The other major groups present were Cirrepede nauplii (3%), Urochordates (2.5%), Polychaetes (1.6%), Decapod larvae (1.5%), and Chaetognaths (<1%). Cladocera (*Penilia avirostris* and *Evadne tergestina)* also made sizable (6%) contribution to the total zooplankton community in January, and were associated with the prasinophyte bloom.

4.3.1.2: HYPOXIC (DO<2 ML L^{-1})

During the SW monsoon, subsurface waters became hypoxic. Waters with DO falling within this range (0.24-1.56 ml l^{-1}; 0.64± 0.39; Table 4A.1) were relatively cool (22.14-26.62 °C; mean ± SD=24.16 ± 1.41). The salinity of such waters varied widely from 22.10 to 35.87 (34.07±4.49). The low salinity values arise from intense rainfall during the SW monsoon and were confined close to sea surface. The upwelled cold, nutrient-rich waters supported high phytoplankton biomass (1.47-7.60 mg Chl *a* m^{-3}; 4.19 ± 2.19). Interesting, the highest chlorophyll concentrations were encountered in near bottom waters at stas. G3 and G5 in September 2005. Maximal contribution (74%) to the total chlorophyll came from pico-autotrophs (<5 - 0.7 μm) with the shares of nano- (<20 - 5 μm) and micro- (>20 μm) fractions being 6.7%

and 18.7% respectively (Fig. 4A.2). Total phytoplankton abundance in hypoxic waters ranged from $1.8x10^4$ to $1.3x10^5$ ($5.9 x10^4 \pm 4.1 x10^4$) cells l^{-1}, consisting of 68 species (56 species of diatoms and 12 species of dinoflagellates) belonging to 36 genera (Table 4A.2). Species diversity and evenness were relatively higher than in oxic water (Table 4A.3). Major diatom species present were *Thalassiosira subtilus, Skeletonema costatum, Leptocylindrus minimus, Cheatoceros spp., Rhizosolenia setigera* (centric forms); *Thalassionema nitzschoides, Thalassiothrix* spp., *Pseudo-nitzcshia* spp., *Asterionella japonica, Navicula transitrans* var. *derasa* f. *delicatula* and other *Navicula spp.* (pennate forms). The endemic species of hypoxic environment are shown in the dendogram (see below). Interestingly, some of the diatoms (*Planktonella sol, Triceratium* spp., *Raphoneis, Coconeis* spp., *Amphora* spp., *Melosira borealis*) and dinoflagellates (*Dinophysis acuminate, Heterocapsa* sp., *Polykrikos* and *Pyrophacus horologium*) that were present in oxic waters were absent in hypoxic waters. The dinoflagellates numbered between 100 and 500 cells l^{-1} and accounted for <5% of the total phytoplankton in hypoxic waters. Of these *Prorocentrum dentatum, Gymnodinium* spp., *Gyrodinium* spp., *Ceratium furca, Noctiluca scintillans* and *Protoperidinium* spp. were commonly recorded.

A change in the mesozooplankton community structure was also observed in hypoxic waters. Their abundance (259-13312; 3190 ± 5673 org. m^{-3}) and biomass (5.3-100; 32 ± 39 ml 100 m^{-3}) were relatively lower than in oxic waters (Table 4A.1). The contribution of copepods to the total mesozooplankton abundance was also lower (77%) as compared to oxic waters. *Acartia erythraea, Acartia spinicauda, Acartia amboinensis,* and *Paracalanus* spp. were the predominant species (Table 4A.4). The other groups present were Decapod larvae (*Lucifer hensani*) (10%), Cladoceran (7%), fish eggs (2%) and Appendicularian (2%). Unlike the phytoplankton community, zooplankton diversity and species richness were the least in hypoxic environment (Table 4B.3).

4.3.1.3: SUBOXIC (<0.2 ml L^{-1})

The suboxic condition prevailed during August-October with the observed DO concentrations ranging between 0.03 and 0.13 ml l^{-1} (0.08 ± 0.033). These concentrations are very close to the detection limit of the Winkler procedure. The suboxic water had low

temperatures, ranging from 22.52 to 25.23 °C with a mean (±SD) of 23.63 ± 0.93 °C reflecting their deeper, offshore origin, while the salinity varied from 32 to 36 (34.3 ± 1.5), the lower values ostensibly caused by some mixing with the fresher surface layer. Phytoplankton biomass varied between 0.38 and 8.51 mg Chl *a* m^{-3} (3.04±2.61) (Table 4A.1). The lowest chlorophyll concentration was recorded in August 2005 at stn. G5 (19 m) whereas the highest concentration also came from subsurface (stn. G5, bottom) in September 2005. The size-fractionated chlorophyll data showed larger biomass in the pico- fraction (66%) than in the nano- (8.8%) and micro- (24.7%) fractions (Fig. 4A.2). Overall, phytoplankton density ranged from $6.4x10^3$ to $7.2x10^4$ ($3.4 \pm 2.2 x10^4$) cells l^{-1} belonging to 52 species (47 species if diatoms and 5 species of dinoflagellates). The dominant species associated with suboxic waters were *Thalassiosera subtilus, Leptocylindrus minimus, Cheatoceros spp., Skeletonema costatum, Rhizosolenia* spp, *Guinardia striata* (centric diatoms), and *Pseudo-nitzschia* spp., *Thalassionema nitzschoides, Thalasiothrix* spp., *Asterionella japonica* and *Navicula transitrans* var. *derasa* f. *delicatula* (pennates) (Table 4A.2). A species of *Gyrodinium* was the only major dinoflagellate found in suboxic waters (density >100 cells l^{-1}). Other less abundant diatoms and dinoflagellates were *Hemiaulus sinensis, Rhizosolenia setigera, Pleurosigma elongatum, Actinoptichus undulates, Cosinodiscus radiatus, Corethrone hystrix, Cocconeis* sp., *Ditylum sol, Ditylum brightwelli, Dactyliosolen* sp., *Eucampia zodiacus* (diatoms) and *Ceratium fusus, Gonyaulax* sp., *Protoperidinium* and *Scrippsiella trochoidea* (dinoflagellates).

The zooplankton density varied from 303 to 1724 (962±550) org m^{-3} and biomass was also low (5.3-27.6 with a mean ± SD of 16±8.7 ml 100 m^{-3}). The zooplankton community in suboxic waters was also dominated by Copepoda (81%) with *Acartia spinicauda, Acartia amboinensis, Paracalanus* spp., *Centropages trispinosus,* and *Centropages tenuiremis* being the major species. Other major (>2%) groups were Decapods (8%), Cirrepede nauplii (5%) and Cladocera (2%). Larval forms of fish, Gastropods, Peleycepod, Pteropods and Polycheates were absent in suboxic waters (Table 4A.4).Phytoplankton diversity and evenness in suboxic waters were comparable to those in hypoxic waters, whereas in the case of mesozooplankton these indices were higher (Table 4A.3A and B).

4.3.1.4: ANOXIC (~0 ml l^{-1})

In the fall intermonsoon phase, particularly in October 2006, near-bottom waters often became completely anoxic. These waters were characterized by low temperatures (23.3 - 23.6; 23.5±0.14°C) and high salinity (mean 35.48) (Table 4A.1). Chlorophyll *a* varied between 1.11 and 3.84 (2.58±1.38) mg m^{-3}, with the pico-autotrophs constituting about 68% of the total phytoplankton biomass (Fig. 4A.2). Even at undetectable levels of dissolved oxygen, some algal forms were present, and their abundance varied between $5.6x10^3$ and $8.2x10^4$ ($3.8 \pm 3.9 x10^4$) cells l^{-1}.

Some of the dominant species found in anoxic waters were *Thalassiosira subtilus, Melosira* spp., *Leptocylindrus minimus, Rhizosolenia setigera* and *Actinoptichus undulates* (centric diatoms); and *Thalassionema nitzschoides, Pseudo-nitzschia* spp., *Thalassiothrix* sp., *Navicula transitrans* var. *derasa, N. transitrans* var. *derasa* f. *delicatula, Pleurosigma angulatum, Pleurosigma elongatum, Pleurosigma* spp. (pennate diatoms). The contribution of pennate diatoms to the total abundance was three fold higher than that of centric forms. Composition-wise the latter forms were more diverse. Interestingly, the dinoflagellate community was virtually absent except for *Gyrodinium* species (Table 4A.2). Species diversity in anoxic waters was also not much different from that in the oxic environment (Table 4A.3A).

As near-bottom waters became anoxic, the effect was observed on the zooplankton community living in the water column. The abundance and biomass were much lower than in other environments: 36-1510 (750±738) org m^{-3} and 0.9-20 (12±9.9) ml 100 m^{-3}, respectively. The dominant group (Copepoda, 82%) mostly comprised of herbivores and omnivores such as *Acartia spinicauda, Acartia amboinensis, Paracalanus* spp., *Centropages trispinosis,* and *Centropages tenuiremis*. This was followed by Decapoda that accounted for 6.5% of the total zooplankton community. Cladocera (*Evadne tergestina*, 8% and *Penilia avirostris*, <1%) were present during this period but mainly close to the surface. On the other hand, Cnidarians, polychaetes, amphipods, ostracods, salps, and other copepods like *Acartia negligens, Undinula vulgaris, Pseudodiaptomus serricaudatus, Pontenella* spp., *Candacia* spp. and *Calanopia* sp.

present under other conditions were absent in anoxic waters (Table 4A.4); species diversity was also low (Table 4A.3B).

Overall, as DO concentrations decline, both phytoplankton and mesozooplankton abundances and their standing stocks also exhibit marked decreases (Fig. 4A.3).

Cluster analysis of phytoplankton composition at 60% similarity showed 5 distinct clusters (Fig. 4A.4A). Clusters A and C contain species that thrive exclusively in oxic and hypoxic waters, respectively. Clusters B and D include species that can tolerate a wide range of oxygen levels (from oxic to suboxic) although they are distinctly separate in terms of their abundance and feeding habits. Cluster B mainly comprises the mixotrophic group while Cluster D contains mostly the autotrophic forms. Finally, Cluster E includes cosmopolitan species present throughout the year irrespective of the oxygen level.

Similarly, an overview of zooplankton community structure is shown in Fig. 4A.4B (dendogram at 60% similarity). This divides pelagic zooplankton into 4 clusters. Cluster A divides into two subgroups A1 and A2 that contain species present in suboxic and anoxic waters, respectively. Cluster B contains species that thrive exclusively in oxic waters. Cluster C pools species that are found to tolerate a wide range of DO concentrations but abundantly present in oxic conditions containing mixed feeders while D cluster comprises species that are present under all conditions but less abundant in oxic conditions and mostly belonging to omnivory diet.

4.4. DISCUSSION

The study area situated along the central west coast of India experiences large changes in hydrography and circulation which, in turn, cause the development of seasonally contrasting biogeochemical regimes including the prevalence of wide-spread and very severe oxygen deficiency during late summer and early autumn. The results obtained in the present study are consistent with this now well - established trend of variability of physico - chemical parameters. Additionally, we observed large shifts in the abundance and composition of both phyto- and zoo-plankton that can be related to changes in ambient DO concentrations.

The establishment of the SW monsoon circulation makes a profound impact on the chemistry of waters over the western Indian continental shelf. The upwelled water is initially hypoxic and rich in macronutrients (nitrate and phosphate). However, the DO in this water gets fully consumed quickly, making the environment reducing. Under these conditions, nitrate is converted to elemental nitrogen by heterotrophic bacteria. This process (denitrification) restricts the availability of bioavailable nitrogen. However, once suphidic conditions develop in near-bottom water, which normally happens over the inner shelf in September-October, nitrogen released from the decaying organic matter accumulates as ammonium (Naqvi *et al.*, 2000; 2006). This progression from hypoxic to anoxic conditions with time is expected to affect the growth and composition of both phyto- and zoo-plankton. Changes in quantity and quality of nutrients are known to influence phytoplankton composition (Hutchings *et al.*, 1995). It is known that large diatoms often predominate in upwelling systems (Malone, 1980; Habeebrehman *et al.*, 2008). These diatoms usually use nitrate. In our study region, however, the above-mentioned shift from nitrate as the dominant form of fixed nitrogen during the early stages of upwelling to ammonium, when the subsurface waters become fully anoxic toward the end of the upwelling period, is expected to affect the phytoplankton composition. Moreover, as the phytoplankton also require oxygen for their own metabolism, completely anoxia may not be suitable for their growth despite the availability of macronutrients (Naqvi *et al.*, 2010). Our size-fractionated Chl *a* data show that during the NE monsoon and spring intermonsoon periods, when oxic conditions prevail throughout the water column, microplankton account for roughly 50% of the total biomass, thereby playing a key role in food web dynamics (Fig. 4A.2). Unfortunately, we do not have any data from early and mid-monsoon phases (June-July) when nitrate supply is high and oxygen-deficiency is not so severe, but the work of Habeebrehman *et al.*, (2008) from the southeastern Arabian Sea shows high abundance of diatoms such as *Skeletonema costatum*, *Rhizosolenia setigera* and *R. alata* during this period (it may be noted that the oxygen-deficiency is also less severe in this area as compared to our area of observations). Likewise, *Nitzschia delicatissima*, *Rhizosolenia styliformis*, among other diatoms, have been found to be characteristic species in the Somali upwelling zone (Smith and Codispoti, 1980). Pigment measurements also showed that next to Chl *a* fucoxanthin, the marker pigment of diatoms is usually the most abundant phytoplankton pigment in the Arabian Sea during the SW monsoon (Latasa and Bidagare, 1998; Roy *et al.*, 2006). In a more recent study of pigments at the CaTS

location, Roy (2010) found that as the nitrate gets consumed, presumably by both autotrophs and heterotrophs, the dominance of diatoms and dinoflagellates decreases, and the contribution of small green flagellates and cyanobacteria increases (Roy, 2010). In conformity with this finding, our results show that in the oxygen deficient waters (hypoxic-anoxic) cells larger than 5 μm in size contributed only 30% to the phytoplankton biomass (Chl *a*) with the bulk (70%) of the biomass occurring in the smaller size range (0.7-5 μm) (Fig. 4A.2). Flow cytometry and HPLC pigment data (not presented here) suggest the dominance of *Synechococcus* in low oxygen waters. However, there are times when the anoxic near-bottom waters have high Chl *a* content, which may be because of sinking of large cells from the surface and their preservation in anoxic waters. Overall, the available data suggest a clear shift in autotrophic community in oxygen deficient waters which seems to be dominated by picoplankton rather than microplankton, mostly diatoms, which account for the bulk of phytoplankton biomass in oxic waters.

Some diatoms have been found to be present throughout the year irrespective of the oxygen level *viz. Leptocylindrus minimus, Thalassiosera* spp., *Skeletonema costatum, Thalassionema nitzschoides, Pseudonitzschia* spp. and *Navicula* spp. These species belong to distinct cluster (dendogram Fig. 4A. 4A). Intriguingly, in anoxic waters the contribution of large pennate diatoms was found to be quite sizable in the present study. How the plankton cope with such low DO levels is something of a mystery. A recent study by Kamp *et al.*, (2011) revealed that benthic diatoms can accumulate large amounts of nitrate and respire it anaerobically via dissimilatory nitrate reduction to ammonium (DNRA) during dark or anoxic conditions. Whether such a respiratory pathway also exists in the pelagic diatom community still remains to be demonstrated.

Although the impact of hypoxia on benthic communities has been studied intensively, less is known about its effect on pelagic communities in coastal ecosystems. Water column hypoxia is known to alter the development (e.g. physiology and life cycle), recruitment, patterns of species distribution and migration (Ekau *et al.*, 2009). Given the shallow depths of our sampling sites, the zooplankton sampling in our study was carried out by vertical haul of net covering the entire water column. Thus, unlike some previous studies (e.g. by Roman *et al.*, 1993) in the Chesapeake where a submersible pump was used for zooplankton sampling from

low oxygen waters, our samples would include zooplankton from the oxic-surface layer as well. It is possible that the biomass could actually be more concentrated within the surface layer (Madhupratap *et al.*, 1990). Nevertheless, this layer is generally very thin (< 10 m), and so it is reasonable to assume that a single vertical haul would to a large extent represent conditions prevailing in the oxygen-deficient part of the water column. Overall, a declining trend in zooplankton abundance was observed from oxic to anoxic conditions (Fig. 4A.3). Apart from the avoidance of low oxygen waters by the zooplankton (Madhupratap *et al.*, 1990), reduced recruitment as a consequence of egg mortality in the low-oxygen bottom waters could be another factor contributing to this trend (Ambler 1985; Roman *et al.*, 1993). Eggs of copepods (*Acatia clausi* and *A. steuri*) have been reported to sink at the rate of 32-61 m d^{-1} in water having a salinity of 32 and a temperature of 20 °C (Uye, 1980), similar to conditions prevailing in our area of study. Therefore, zooplankton eggs laid in thin surface layer could rapidly sink to the anoxic layer thereby adversely affecting zooplankton recruitment. Few typical estuarine species (*Pseudodiaoptomus aurivilli, Tortanus forcipatus* and *Metacalanus aurivilli*) that were caught near the mouth of the Zuari estuary were also found to be associated with hypoxia. This estuary is also penetrated by low oxygen bottom water from the sea during the late SW monsoon (Sankaranarayanan and Jayaraman, 1972).

4.5: CONCLUSIONS

Seasonally reversing circulation along the west coast of India results in the prevalence of contrasting biogeochemical conditions in winter and summer, including widespread and severe oxygen deficiency in sub-pycnocline waters during the latter season. These conditions profoundly influence the dynamics of both phyto- and zoo-plankton. While microplakton (mostly diatoms but also dinoflagellates) constitute the major part of the phytoplankton biomass in oxic waters; pico-autotrophs, and to a smaller extent, pennate diatoms are found to be predominant in oxygen deficient waters. The zooplankton biomass is generally higher in oxic waters as compared to oxygen-deficient waters. Among various zooplankton, several species of copepods can survive at low concentrations of oxygen (<1 ml l^{-1}), as revealed by both field data and results of laboratory experiments (see Chapter 7).

Table: 4A.1. Variations of chlorophyll *a* (chl *a*; mg m^{-3}), phytoplankton abundance (phyto; cells l^{-1}), mesozooplankton biomass (zoo-biomass; ml 100 m^{-3}) and abundance (zooplk; ind m^{-3}), temperature (temp; ^0C) and salinity for different categories of oxygen deficiency.

	Parameters	min	max	avg	stdev
OXIC	Temp	26.95	29.19	27.86	0.901
	salinity	34.98	35.79	35.24	0.35
	oxygen	3.13	6.67	4.089	0.966
	chl a	0.68	16.02	4.455	4.457
	Phyto	40000	553333	120766	156219
	zoo-biomass	3.1	266.7	107	128
	zooplk	385	15061	4569	7048
HYPOXIC	Temp	22.14	26.62	24.16	1.41
	salinity	22.1	35.87	34.06	4.49
	oxygen	0.24	1.56	0.64	0.39
	chl a	1.47	7.6	4.185	2.185
	Phyto	18204	135150	54319	41069
	zoo-biomass	5.3	100	32.1	39
	zooplk	259	13312	3190	5673
SUBOXIC	Temp	22.52	25.23	23.629	0.93
	salinity	31.98	36	34.35	1.53
	oxygen	0.03	0.13	0.081	0.033
	chl a	0.38	8.51	3.036	2.613
	Phyto	6429	72660	34246	22865
	biomass	5.3	27.6	16	8.7
	zooplk	303	1724	962	550
ANOXIC	Temp	23.29	23.55	23.45	0.14
	salinity	35.2	35.67	35.48	0.24
	oxygen				
	chl a	1.11	3.84	2.583	1.378
	Phyto	5600	82187	38319	39492
	biomass	0.9	20	12.1	9.9
	zooplk	36	1510	750	738

Table: 4A.2. Phytoplankton composition and abundance for different categories of oxygen deficiency.

Phytoplankton cell L^{-1}	OXIC 120766	HYPOXIC 54319	SUBOXIC 34246	ANOXIC 38319
DIATOM (centric)				
Actinoptychus undulates		3	1	3
Bellerochea horologicalis		2		
Bacteriastrum hyalinum		1		
Coscinodiscus marginatus	2	2		
Coscinodiscus radiates		1	2	
Coscinodiscus spp.	2	2	2	2
Corethron hystrix	1	2	1	
Chaetoceros affinis	1	3		
Chaetoceros lorenzianus	3	2	2	
Chaetoceros curvisetus	6	3		
Chaetoceros decipiens	2			
Chaetoceros spp.	6	4	5	
Cyclotella spp		1	1	
Ditylum sol	2	1	2	
Ditylum brightwellii	2		1	
Dactyliosolen spp		2	2	
Eucampia cornuta	2	2		
Eucampia zodiacus	2	2	2	2
Guinardia striata	4	3	3	2
Guinardia delicatula			1	
Guinardia flaccid			2	
Hemiaulus hauckii	2	1		
Hemiaulus membranaceus		2		
Hemiaulus sinensis		2	3	
Hemiaulus sp		1		
Lauderia sp	2	3		
Leptocylindrus minimus	4	5	5	4
Leptocylindrus danicus	2	2	2	
Odontella mobiliensis	2	1	1	
Odontella sinensis	2	2	2	1
Melosira borreri	2			
Melosira sulcata		1		
Melosira sp	2			3
Planktoniella sol	2			
Rhizosolenia alata		1	2	

Rhizosolenia crassispina				2
Rhizosolenia imbricata var shrubsolei		3		
Rhizosolenia setigera	2	4	2	4
Rhizosolenia stolterforthii		2	2	
Rhizosolenia syliformis		3	2	1
Rhizosolenia spp.	3	2	3	
Schroederella spp		1		
Skeletonema costatum	6	6	5	3
Streptotheca tamesis	3		1	
Thalassiosira subtilis	4	6	6	5
Thalassiosira spp	4	1	2	4
Triceratium sp	1			
DIATOM (pennate)				
Amphiphora spp	1			
Asterionella japonica	2	4	4	
Cocconeis spp.	4		1	
Meuniera membranacea		2		3
Fragilaria oceanic			2	
Navicula transitrans var.derasa	1	2	2	4
N. transitrans var.*derasa* f. *delicatula*	1	5	5	5
Navicula vanhoeffenii		3		
Navicula spp.	4	4	3	3
Nitzschia closterium	1	3	2	
Nitzschia longissima		2		1
Nitzschia sp	2	2	1	2
Pleurosigma directum	2	2	2	2
Pleurosigma angulatum	2	3	2	4
Pleurosigma elongatum	1	3	3	5
Pleurosigma normanii	1			
Pleurosigma spp.	2	3	3	4
Pseudo-nitzschia spp	4	4	4	5
Pseudo-nitzschia seriata	3	3	2	
Gyrosigma balticum		1	2	3
Raphoneis sp	1			
Synedra sp			1	2
Thalassionema nitzschioides	6	6	4	6
Thalassionema frauenfeldii	2	3	2	
Thalassiothrix spp		5	4	4
DINOFLAGELLATES				
Amphidinium spp.	3	1		
Ceratium furca	2	2		
Ceratium fusus		1	1	

Ceratium kofoidii	1			
Dinophysis acuminate	2			
Gonyaulax spp			1	
Gymnodium spp.	4	2		
Gyrodinium spp	4	2	2	1
Heterocapsa spp	5			
Noctiluca miliaris		2		
Prorocentrum micans	2	1		
Prorocentrum dentatum	5	4		
Prorocentrum gracile	3			
Protoperidinium steinii	2	1		
Protoperidinium pentagonum		1		
Protoperidinium depressum	2	1		
Protoperidinium spp.	2	1	1	
Pyrophacus horologium	1			
Polykrikos spp	1			
Scrippsiella trochoidea	6		1	
SILICOFLAGELLATE				
Dictyocha fibula	2			
Dictyocha speculum				1
Ebria tripartite	1			
PRASINOPHYCEAE				
Bloom-Prasinophytes	6			

Note: Range represented as following digits 5 -100= 1; 100-500= 2; 500-1000= 3; 1000-2500= 4; 2500-5000= 5; >5000= 6 cell countsL^{-1}

Table: 4A.3. Species diversity indices for (**A**) phytoplankton and (**B**) zooplankton for different categories of oxygen deficiency : number of species (S); total individuals (N); species richness (d); Pielou's eveness (J'); species diversity H'(loge) at the CaTS site.

(A)

Oxygen level	S	N	d	J'	H'
Oxic	66	120766	5.55	0.66	2.77
Hypoxic	68	54319	6.15	0.77	3.26
Suboxic	52	34246	4.88	0.79	3.11
Anoxic	30	38319	2.75	0.81	2.76

(B)

Oxygen level	S	N	d	J'	H'
Oxic	72	456893	5.45	0.65	2.78
Hypoxic	44	319040	3.39	0.54	2.03
Suboxic	42	96181	3.57	0.69	2.59
Anoxic	45	75000	3.92	0.56	2.13

Table: 4A.4. Mesozooplankton composition and abundance for different categories of oxygen deficiency.

Zooplankton composition (org m^{-3})	OXIC 4569	HYPOXIC 3190	SUBOXIC 962	ANOXIC 750
HYDROZOA				
Siphonophorae	4			
Bassia spp	3			
Diphyes spp	3	4	2	1
Chelophysis spp			4	
Medusae	5		4	1
GASTROPODA	3			1
PTEROPODA				1
POLYCHEATA	6	2	1	
CLADOCERA				
Evadne tergestina	6	6	5	6
Penilia avirostris	2			2
CIRRIPEDIA				
Cirripede nauplii	6	3	6	1
Cirripede cyprius	4			
CALANOIDA				
Acartia erythraea	4	6		2
Acartia negligens	6	2	2	
Acartia spinicauda	6	6	6	6
Acartia amboinensis	2	6	6	6
Acartia centrura	6			
Acartia danae		4	2	
Acartia spp	6	6	6	6
Paracalanus parvus	1	3	3	1
Paracalanus aculeatus	1	1	1	1
Paracalanus sp.	6	6	3	4
Acrocalanus gibber	5	4	5	1
Acrocalanus longicornis		2	2	
Acrocalanus gracilis		1	1	
Acrocalanus spp	5	3	3	2
Calanus tenuicornis	1			
Temora turbinate	5			
Temora discaudata	5			
Temora stylifera	4			
Undinula vulgaris	6	3	3	

Subeucalanus monachus	4			1
Subeucalanus subcrassus.			3	
Subeucalanus pileatus	5		2	2
Canthocalanus pauper	6	1	1	2
Clausocalanus sp	1	1		1
Cosmocalanus darwinii	5			
Centropage furcatus	3			
Centropages orsanii	6			1
Centropages calaninus	5			
Centropage tenuiremis	6	5	6	5
Centopages trispinosus	3	5	6	6
Centropages alcocki	2	3	4	
Centropages spp	1	5	6	4
Pseudodiaptomus serricaudatus	5	3		
Candacia bradyi	3			
Candacia spp	2			
Labidocera minuta	6			
Labidocera pavo	4	2	3	
Labidocera acuta	6	2	2	1
Labidocera pectinata	1	4		
Labidocera sp.	6	4	4	1
Pontenella spp		1	1	
Calanopia eliptica	4			
POICILOSTOMATATOIDA				
Corycaeus catus	3			1
Corycaeus speciosus	2			1
Corycaeus sp.	6	5	2	1
Oncea venusta				1
Oncaea sp.	6			2
Sapphirina nigromaculata	4			
CYCLOPIODA				
Oithona plumifera	5			2
Oithona similes	6			
Oithona spinirostris	2			1
Oithona rigida	6			
Oithona sp.	6	3	3	2
HARPACTICOIDA				
Euterpina acutifrons	5	1	1	1
Microsetella rosea	1	1		
Microsetella norvegica	1	1		
Bomolochus sp.	1	2		

CALIGOIDA	1			
AMPHIPODA	3	2		
OSTRACODA	5	2	2	
DECAPODA				
Decapod larvae	5	6	4	4
Squilla sp	4	2		
Brachyuran zoea	5	5	5	3
Megalopa		1		2
Porcellanid larva	3			
Lucifer hensani		6	5	5
Lucifer typus			2	4
Lucifer sp.	4	2	5	1
CHEATOGNATHA				
Sagitta enflata	2	1	1	2
Sagitta bedoti	5			
Sagitta sp	4		3	3
UROCHORDATA				
Oikiopleura sp.	6	2	2	3
Doliolum sp.	2			1
Salp sp			4	
CHORDATA				
Fish eggs	4	6	3	3
Fish larvae	1			
Unidentified	5			
Total org 100m^{-3}	456893	319040	96181	75000

Note: Range represented as following digits: 5-100=1; 100-500= 2; 500-1000=3; 1000-2500=4; 2500-5000=5; >5000=6 individuals 100m^{-3}

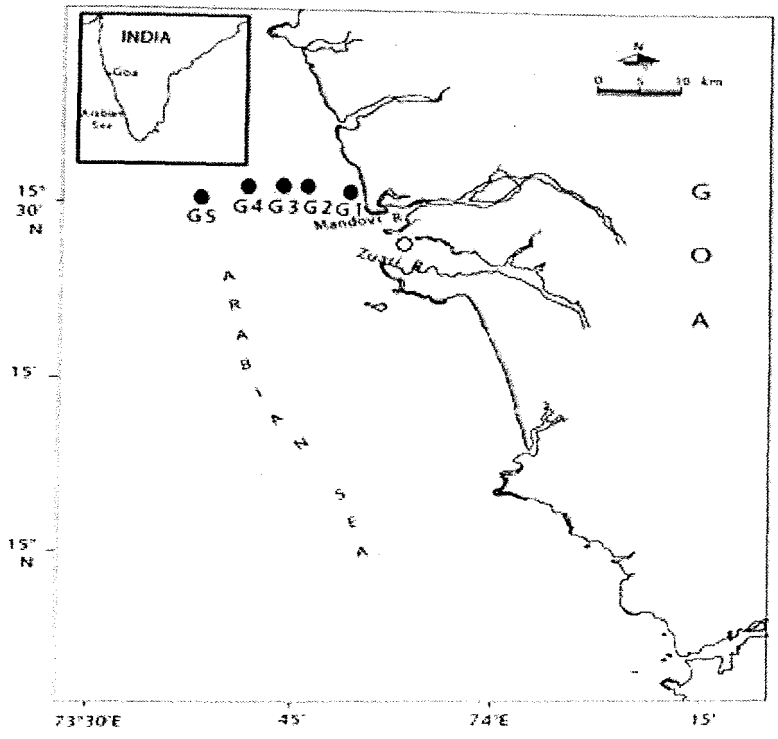


Fig. 4A.1. Map showing study area and stations (G1-G5, closed circles) that form the CaTS transect. Stn Z1 (open circle) is the sampling site for collection of zooplankton for experimental purpose.

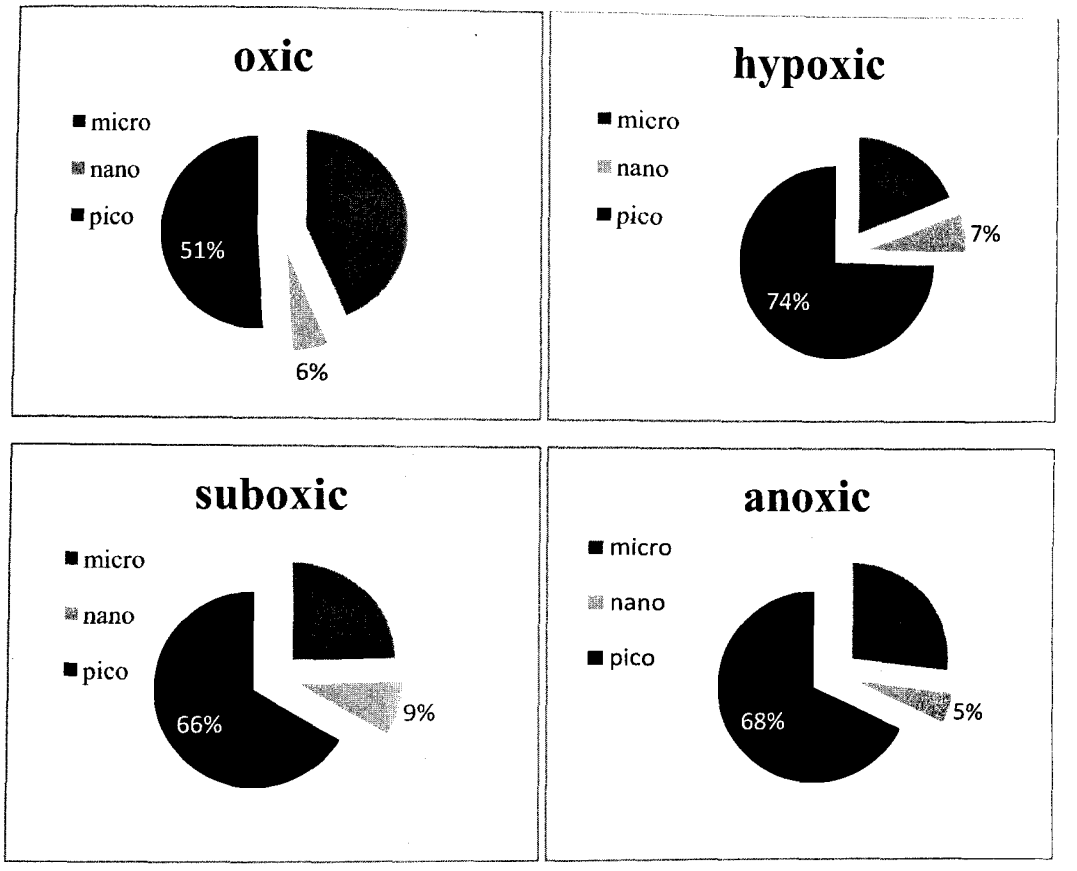


Fig. 4A.2. Size- fractionated Chl *a* distribution representing abundance of of pico (<10-0.7μm); nano (<20-10 μm); and micro plankton (>20 μm) in the study area under (**A**) oxic; (**B**) hypoxic; (**C**) suboxic and (**D**) anoxic conditions for the years 2005-2006.

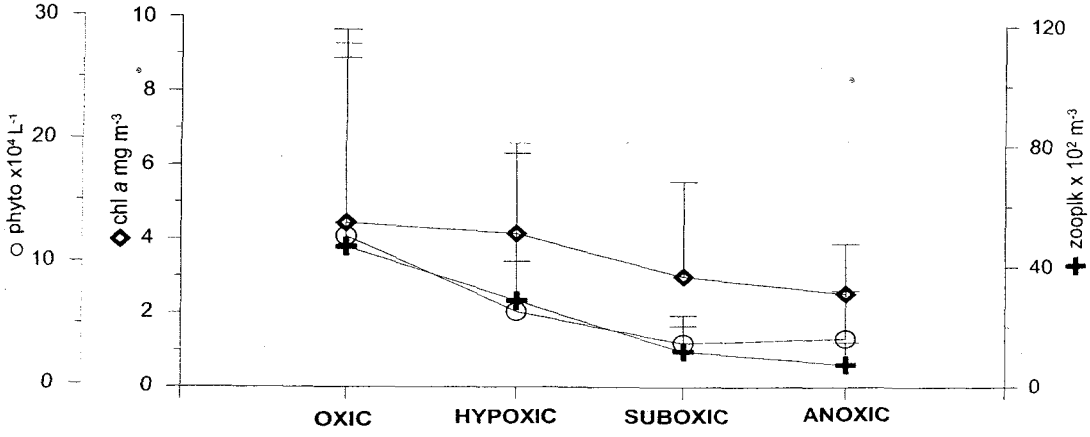


Fig. 4A.3. Average variations in abundance (phytoplankton and zooplankton) and biomass (Chl *a*) from oxic to anoxic conditions.

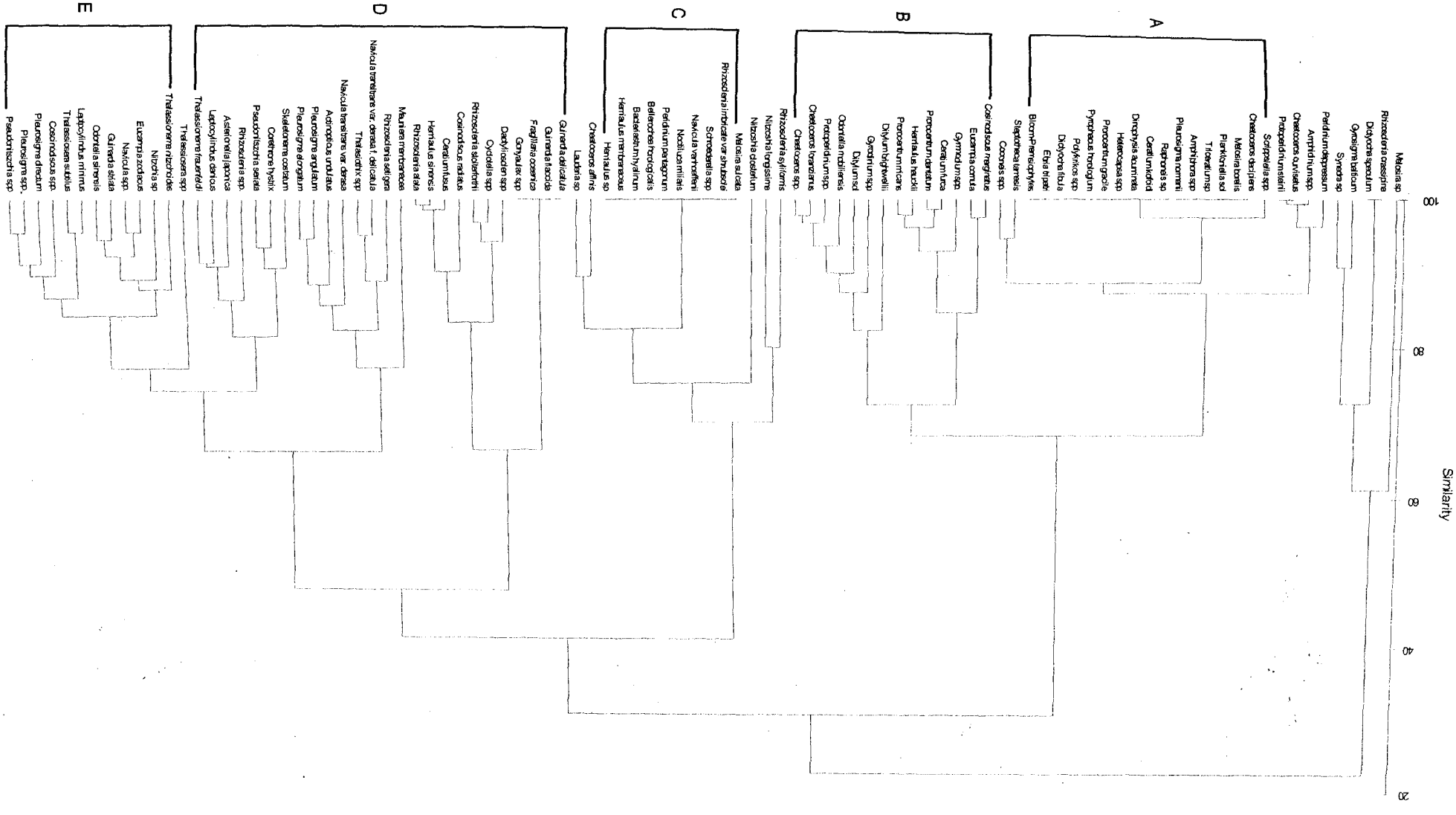


Fig. 4A.4A. Dendogram based on Bray-Curtis similarity showing average linkage cluster analysis for phytoplankton taxa in oxic , hypoxic , suboxic and anoxic waters.

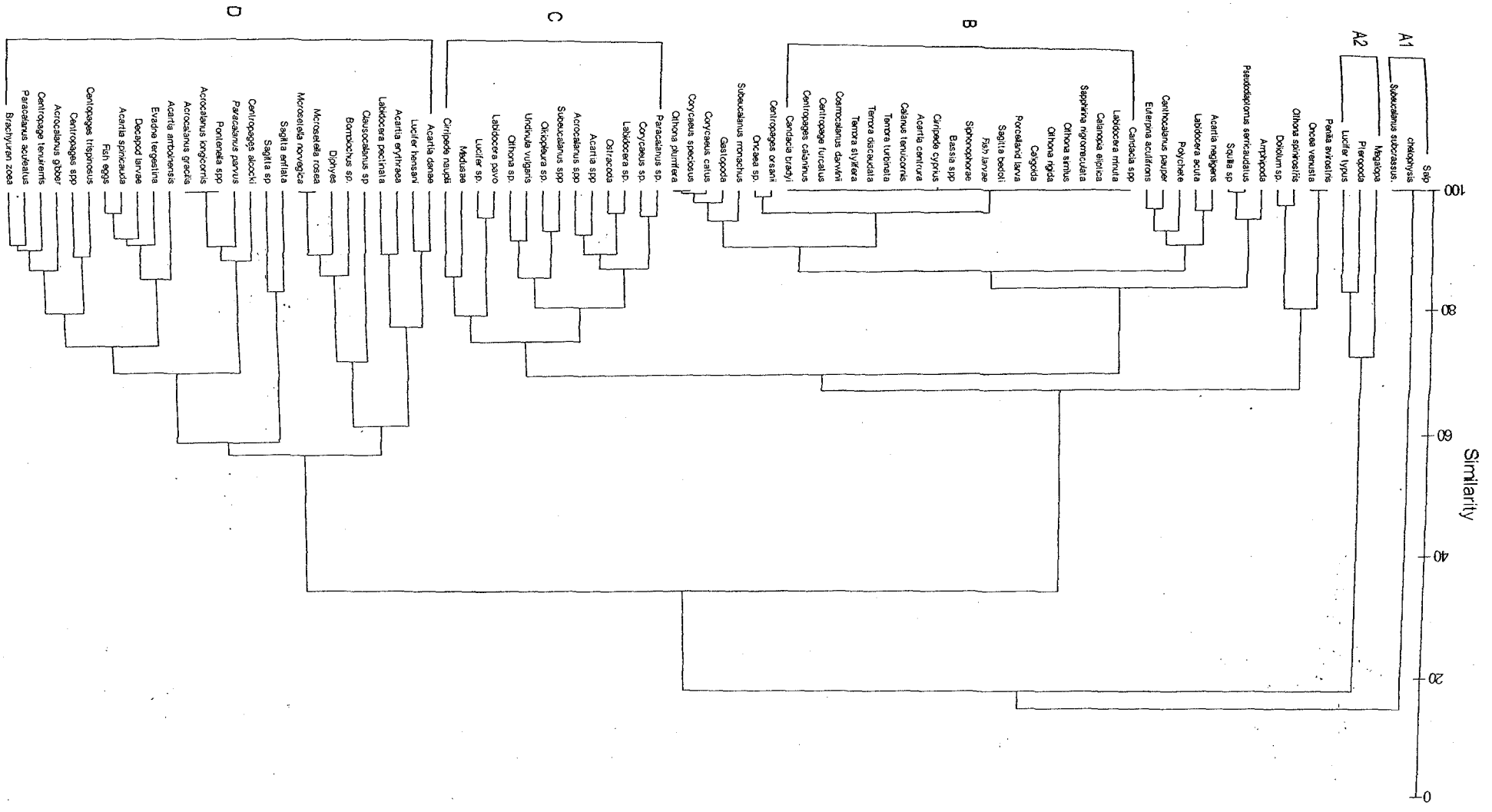


Fig. 4A.4B. Dendogram based on Bray-Curtis similarity showing average linkage cluster analysis for zooplankton taxa in oxic , hypoxic , suboxic and anoxic waters.

SECTION-4B

SEASONAL AND INTERANNUAL VARIATION OF THE PHYTOPLANKTON AND MESOZOOPLANKTON IN COASTAL WATERS OF GOA, INDIA

RESULTS

VARIATIONS IN BIOLOGICAL PARAMETERS AT THE CaTS SITE: YEAR 2005

4B.1: CHLOROPHYLL A (Chl *a*):

On an annual scale, phytoplankton biomass (chl *a*) at different CATS location (G1-G5) in 2005 varied between 0.19 and 17.7 mg m^{-3} (Avg 2.57± 3.16 mg m^{-3}) (Fig. 4B.1) Chlorophyll *a* concentration was high at # G1 particularly during September and least at stn G3, February '06. However, thick bloom of unidentified photosynthetic forms was also recorded at station G1 during the month of January'05, which had chlorophyll *a* concentration as high as 107 mg m^{-3} followed by a second peak in September with 17.7mg m^{-3}. The annual chlorophyll distribution at stn G1, G2, G3, G4 and G5 are shown in the Fig. 4B.2a,b.

Data has been classified 4 seasons namely as North east monsoom (NEM) December-February); Spring inter monsoon (SIM) April-May); South west monsoon (SWM) June - September) and Fall intermonsoon (FIM) October-November). Seasonally highest (19.9 mg m^{-3}) and lowest (0.74 mg m^{-3}) algal biomass was recorded during NEM at stn G1 and G5 respectively.

However, during NEM chl a ranged from 0.41 mg m^{-3} (Jan, G4) and 54.6 mg m^{-3} (Jan, G1), Average (5.47±13.8 mg m^{-3}), during SIM ranged from 0.68 mg m^{-3} (May, G5) and 4.9 mg m^{-3} (May ,G1), Average (2.27+1.27 mg m^{-3}); SWM ranged from 2.77 mg m^{-3} (Aug, G5) and

12.68 mg m^{-3} (Sep, G1) Average (5.48±3.77 mg m^{-3}), and in FIM ranged from 0.80 mg m^{-3} (Nov, G5) and 1.8 mg m^{-3} (Oct, G1); Average (1.31± 0.40 mg m^{-3}) (Fig. 4B. 1b). Further, spatially chl a concentration was highest at stn G1 (avg. 10.5±18) and lowest at Stn G4 (avg 1.4±0.9). A general overview shows that phytoplankton biomass shows a decreasing trend towards the deeper stations like G4-G5. (Fig. 4B.2a)

4B.2: PHYTOPLANKTON

Phytoplankton abundance at different CaTS location (G1-G5) varied between 0.15-14177 x10^3 cells l^{-1} (avg.165±1331 x10^3). Overall, peak abundance was recorded particularly at # G1 during January and then subsequently in September, the former, was due to the predominance of unidentified spherical green cell (~5um) belonging to class *Prasinophyceae* (81%) with cell counts as high as 1150000 L^{-1} along with other dinoflagellates (>17%) and later bloom was of *Prorocentrum sp* which alone contributed to (92%). While the least abundance was recorded in the month of February at mid depth of stn G3 (7m). The annual phytoplankton abundance at stn G1,G2,G3,G4 and G5 are shown in the Fig. 4B.3a and b.

Seasonally highest cell density (3667 x 10^6 cells L^{-1}) was recorded during SWM at stn G1 and least (6.2 x 10^6 cells L^{-1}) was recorded during NEM at stn G5 (Fig. 4B.2a.) During NEM phytoplankton density ranged from 0.5 x 10^6 (Feb, G3) and 728x10^6 (January, G1), average (68 ± 183 x 10^6 L^{-1}). During SIM ranged from 37 x 10^6 (April, G5) and 128 x 10^6 (May, G1), average (64±30 x 10^6 L^{-1}). In SWM ranged from 36 x 10^6 (Aug, G1) and 7297 x 10^6 (Sep, G1) average (1265 ± 2955x 10^6 L^{-1}), and during FIM ranged from 6 x10^6 (Nov, G5) and 38 x10^6 (Oct,G1); average (19±10 x 10^6 L^{-1}). Similar to chlorophyll a pigment, cell abundance also remained low towards the offshore stations (Fig. 4B.3b).

4B.2.1: PHYTOPLANKTON COMPOSITION

Total 108 species of phytoplankton were identified of which 77, 28, 1 and 1 species were belonging to diatoms, dinoflagellates, silicoflagellates, Blue green algae and *Prasinophyceae* respectively. Among these four groups diatoms were predominant forms followed by

dinoflagellates, except during the bloom period. On an average diatom and dinoflagellates contributed 75% and 16% respectively. Contribution of silicoflagellates and other forms such as blue green algae were generally insignificant (<2%) during the most part of the year. During, the blooms phase of Prasinophyte other algal groups such as dinoflagellates (*Scrippsiella trochoidea, Prorocentrum dentatum* and *Heterocapsa* sp.) also proliferated. While a single dinoflagellate bloom of *Prorocentrum dentatum* occurred in the month of September.

Of the 38 diatom genera, the most commonly found were *Chaetocereos,Coconeis, Coscinodiscus, Ditylum, Guinardia, Navicula, Nitzschia, Thalassionema, Leptocylindrus, Skeletonema, Rhizosolenia, Thalassiosera, Melosira, Pleurosigma,, Ditylum, Eucampia, Odontella, Asterionella* and Amongst dinoflagellates,*Amphidinium, Prorocentrum, Ceratium,, Gyrodinium, Protoperidinium, Gymnodinium, Pyrophacus, Dynophysis* and *Gonyaulax* were common of the 15 identified genera. A few representative species are also shown in (Plate I-VIII.) Among silicoflagellates, *Dictyocha* spp was quite common. The comparative list of total phytoplankton community is presented in the Table .1.

4B.2.2: PHYTOPLANKTON PRODUCTIVITY (PP)

On an annual basis, the surface PP values ranged between 0.2 to as high as 572 mg C m^{-3} d^{-1}. This high productivity rates was recorded in surface waters of stn G5 in the month of April. Standing stock of phytoplankton biomass (chl a) was also large (6.7 mg m 3) during this period. Likewise, high surface PP was also recorded at stn G1 and G3 with >340mg C m^{-3} d^{-1}. It was interesting to find PP rates as low as 0.2 mg C m^{-3} d^{-1} at mid depth (ca. 18 mts of stn G5) in the month of September. Decreased PP rates in the shallow waters rich in nutrient (and light) during late southwest monsoon possibly due to the prevailed critically low oxygen (0.6ml L^{-1}) water at this depth even though chlorophyll biomass was sizably large (ca.>4 mg m^{-3} ; Fig. 4B.4). A similar trend was also observed at stn G1 (surface) in the month of August with low PP (2.4 mg C m^{-3} d^{-1}) associated with high chlorophyll a stock (>4.6 mg m^{-3}). Overall, column production (stn G5; 0-27m) was found increase as high as 3.5 g C m^{-3} d^{-1} in December. As expected least rates was recorded duing late south west monsoon (0.3 g C m^{-3} d^{-1}).

Generally, the surface waters of coastal region remains well mixed and oxygenated for most part of the year supporting about 99% PP in the upper 18 m depth and a small (<0.5 %) occurs at near bottom depth (27 m) particularly in the month September during which near bottom waters experience suboxia (0.1 ml L-1) on a seasonal scale. Whereas, during non monsoon months even subsurface waters remains well mixed (oxic waters; >3 ml L^{-1}) supporting a sizable production (ca. 29%) of autotrophs.

4B.3: MESOZOOPLANKTON

Similar to phytoplankton distribution, the abundance and biomass record showed higher values at shallow stations (G1-G2) and decreased towards deeper stations (G4-G5) Fig. 4B.5a. Overall, the abundance at various stations (G1-G5) during the year 2005 (Dec' 04- Nov' 05) varied between 1.4 and 188 $x10^2$ m^{-3} avg. (39 $\pm$ 48.7 $x10^2$ m^{-3}). The highest abundance wad recorded at stn G2 in December'04 and lowest was at stn G4 in November'05 (Fig. 4B.5a and b). While biomass ranged from 3.6 (stn. G2, Jan) to 267 ml m^{-3} (stn G1, May). Station wise, average abundance showed a peak at stn G2 and was least at stn G3 while high and low biomass was recorded at stn G1and G3 respectively (Fig. 4B.5a).

Monthwise, average abundance varied between 4.4 - 88 $x10^2$ m^{-3} (avg.38$\pm$ 30 $x10^2$ m^{-3}). High and low values were reported in the month of October and November respectively (Fig. 4B.5b.) Similarly, its biomass ranged from 10.8 (Jan'05) -103 (February,'05) ml m^{-3} avg.(49.8$\pm$31 ml m^{-3}) (Fig. 4B.5b).

Seasonally, an average value of abundance of mesozooplankton during different seasons such as NEM, SIM, SWM and FIM are 32$\pm$20.5; 43.29$\pm$41.2; 21$\pm$23 and 67.7$\pm$48 $x10^2$ m^{-3} respectively (Fig. 4B.4). On the whole, high abundance was observed during FIM and least was recorded during SWM (Fig. 4A.6). While biomass was highest during NEM (0.67 ml m^{-3}) and lowest was during SWM (0.28 ml m^{-3}) (Fig. 4B.6).

4B.3.1: MESOZOOPLANKTON COMPOSITION

The mesozooplankton composition was mainly dominated by sub class copepod (69%) throughout the region (CaTS location). These copepods mainly belong to 4 orders *viz.* Calanoida, which accounted on an average (57%), Poiceilostomatoida (6%), Cyclopoida (4%) and Harpacticoida with 2%. followed by Cladocera (19%) and Urochordates (7%) while Decapoda, Hydrozoa, Cheatognaths and other groups together contributed to 14% (Fig. 4B.7). Likewise, of the total 91 mesozooplankton species identified, 72 species with 28 genera belonged to copepods. Among the Copepods identified Calanoida were more diverse with 18 genera, while Harpacticoida, Poicilostomatoida and Cyclopoida accounted to 5, 4, 1 genus respectively. The most common copepod species encountered belonged to genus *Acartia, Paracalanus, Centropages, Acrocalanus, Labidocera, Temora, Corycaceus, Oithona, Oncea, Euterpina* (see Plate IX-XI) while others groups were Urochordates (Oikopleura sp); Cladocera (*Evadne tergestina, Penilia avirostris*); Decapoda (*Lucifer hensani, Squilla* sp) and Cheatognaths (*Sagitta betoti, S.enflata*).(see Plate.. XVII)

Seasonally, mesozooplankton distribution did not show much variation and Copepoda dominated throughout the season, however there was a swarm of Cladocera (*Penilia avirostris* and *Evadne tergestina*) prevailing in the month of October'05 and contributed on an average 81% of the total community. During NEM the major groups were copepod, the average percentage is as follows; copepod -78%) followed by Tunicates-13%, Cheatognaths-3%. During Spring Inter-Monsoon (SIM), the dominant groups were Copepods (avg. 76%), Urochordates (9%), Cladocera (7%), During SWM, Copepoda (81%), Hydrozoa (4%) and Decapoda (3%). While in Fall Inter-Monsoon (FIM), Cladocera were the dominant group with 49% followed by Copepoda (33%) and Cheatognaths (4%) Fig. 4B.8.

Mesozooplankton based upon their feeding habitat they are classified into 4 major categories namely: herbivores, carnivores, omnivores and suspension feeders or detritivores. Interestingly, on the whole during NEM and SIM the herbivores dominated while during SWM and FIM omnivores dominated (Fig. 4B.9).

VARIATIONS IN BIOLOGICAL PARAMETERS AT THE CATS SITE: YEAR 2006

4B.4: CHLOROPHYLL A (Chl *a*)

On an annual scale, phytoplankton biomass (chl *a*) at different CATS location (G1-G5) in 2006 varied between 0.15 and 8.15 mg m^{-3} (Avg. 1.86+ 1.66 mg m^{-3}). This high chlorophyll *a* concentration was observed at # G5 subsurface depth in September'06 and least was recorded at in the month of February at near bottom depths of stn G5. The annual chlorophyll distribution at all station from G1 to G5 is shown in the Fig. 4B.10b

Seasonally, highest algal biomass was recorded during SIM (3.14 mg m^{-3}) followed by SWM (2.8 mg m^{-3}) and least was recorded during FIM (1.62 mg m^{-3}) (Fig. 4B.8a). However, during NEM chl ranged from 0.34 mg m^{-3} (Feb'06,G5) and 6.13 mgm^{-3} (Dec'05, G1), average(1.64±1.4mg m^{-3}), during SIM ranged from 1.75 mg m^{-3} (April'06, G2) and 4.7 mg m^{-3} (April ,G5), average(3.14+1.29 mg m^{-3}); SWM ranged from 0.8 mg m^{-3} (Sep'06,G4) and 4.85 mg m^{-3} (Aug'06,G3) average(2.60±1.5 mg m^{-3}), and in FIM ranged from 0.47 mg m^{-3} (Nov'06,G5) and 2.75 mg m^{-3} (Nov'06,G1) average(1.62± 0.7 mg m^{-3}) (Fig. 4B.8a). Thus, SIM and SWM sustained higher chl biomass as seen in the graph (Fig. 4B.10 a and b). Further, spatially chl biomass was highest at stn G1 (avg. 3.1±0.8) and lowest at Stn G4 (avg 1.5±0.7). A general overview of chla also shows a decreasing trend towards the deeper stations like G4-G5 (Fig. 4B.10b).

4B.5: SIZE FRACTIONATED CHLOROPHYLL

Size fractionated chl (>200, 60, 20, 5 and >0.7μm) data has been clustered according to its size class as pico (<5-0.7 μm), nano (<20-5 μm) and micro (>20 μm). Based upon the size fractionated chl a measurement study at different stations from the coast G1 to offshore stn G5, certainly showed that the pico fraction formed the major fraction of phytoplankton biomass (chl *a*) but interestingly the chlorophyll distribution varied in percentage contribution showing relatively high pico biomass at G1 as compared to G3 and G5 (Fig. 4B.9a). Near the coastal region i.e. at G1, pico on an average contributed to 73% alone, while micro and nano fraction

average contribution was 22 and 5 respectively. While, at stn G3 and G5 pico , micro and nano plankton biomass were on an average 65, 29 and 6 respectively (Fig. 4B.11a).

Seasonally, during NEM and SIM pico fraction accounted to <60%, during SWM, 66% while during FIM picoplankton biomass contributed to 81% (Fig. 4B.11b). To summarize the smaller cell size fraction of chlorophyll which is dominant decreases gradually towards off coast and is taken over by the larger forms of phytoplankton and seasonally during FIM pico fraction played a substantial role.

4B.5: PHYTOPLANKTON

Phytoplankton abundance at different CATS location (G1-G5) in 2006 varied between 0.28 to 1672 $x10^3$ cells L^{-1} (avg 59$\pm$ 180 $x10^3$). This peak abundance was recorded in December at #G4, due to dominance of diatoms (*Melosira* sp., *Cheatoceros* sp., and *Pseudonitzschia* sp.).While the least abundance was recorded in the month of February at stn G3 .On the whole stationwise average abundance showed highest cell density at stn G1 (avg.48+28) $x10^3$ L^{-1} and lowest was recorded at stn G5 (avg.28+31)) $x10^3$ L^{-1}. However, since there was no data for SIM at station G1 to G4, station G5 showed higher abundance (Fig. 4B.12a.)

Seasonally, on the whole highest cell density (70.8 x 10^6 cells L^{-1}) was recorded during SIM and least (29.2 x 10^6 cells L^{-1}) was recorded during NEM at stn G5(Fig. 4B.10a and b). The data during SIM was collected only at stn. G5. During NEM phytoplankton density ranged from 16.2 x 10^6 (stn G5) and 54 $x10^6$ (stn G1), average (29+14.8 x 10^6 L^{-1}). During SWM ranged from 16 x 10^6 (stn G5) and 51 x 10^6 (stn G3), average (40 $\pm$14x 10^6 L^{-1}) and during FIM it ranged from 21 $x10^6$ (stn G4) and 43 $x10^6$ (stn G5); average (35$\pm$9.4 x 10^6 L^{-1})(Fig. 4B.12b).

4B.5.1: PHYTOPLANKTON COMPOSITION

A total of 120 species of phytoplankton were identified with 82, 34, 2, 1 and 1 belonging to diatoms, dinoflagellates, silicoflagellates, Blue green algae and Prymnesiophyceae

respectively. Among these four groups, diatoms were predominant forms followed by dinoflagellates. Diatoms on an average accounted 83% as against 15% by dinoflagellates. Contributions of silicoflagellates and other forms such as blue green algae were generally low (<0.2%) during most part of the year. Highest diversity was recorded in the peak SW monsoon (August) with 103 species and least in April with 25 species. A thick bloom of *Trichodesmium erythraeum* was recorded at near surface water of station G6 in the end of February to March'06, resulting chlorophyll *a* concentration as high as 11 mg m^{-3} (data not shown). Other forms such as colonial *Pheocystis* spp (prymnesiophyte-flagellate) were also observed at offshore station in the month of November '06, but their contribution was comparatively low about 7% to the total phytoplankton community

In general, diatom abundance was 2.5 times higher than that of dinoflagellate. Of the 39 diatom genera the most commonly found were *Chaetocereos, Coscinodiscus, Navicula, Nitzschia, Thalassionema, Leptocylindrus, Skeletonema, Rhizosolenia, Thalassiosera, Melosira, Pleurosigma ,Ditylum, Eucampia, Odontella, Asterionella and Guinardia.* Overall centric diatom accounting to ca. 64% are predominant over pennate forms in this study region. Amongst dinoflagellates, *Prorocentrum, Ceratium, Scripsiella, Gyrodinium, Protoperidinium, Gymnodinium, Pyrophacus, Dynophysis and Gonyaulax* were the common genera. Harmful toxic dinoflagellate namely *Cochlodinium catenatum* were also observed during the month of October-November'06. The comparative list of total phytoplankton community present seasonwise throughout the year is shown in the Table 4B.2

4B.6: MESOZOOPLANKTON

Unlike phytoplankton distribution, the mesozooplankton abundance and its biomass record showed higher values at mid station stations (G2-G3) Fig. 4B.11a. Overall, the abundance during the year 2006 (Dec' 05- Nov' 06) varied between 0.7 (stn G1, Oct'06) and 7202 (stn G3, Jan'06) $x10^3$ m^{-3} avg. (396 $\pm$ 1181 $x10^3$ m^{-3}). Similarly mesozooplankton biomass was minimum the month of October (stn G2) and maximum in the month of January (stn. G2). Station wise, average abundance showed a peak at stn G3 and was least at stn G4 while high and low biomass was recorded at stn G2 and G5 respectively (Fig. 4B.13a). Monthwise, average abundance varied between 3 x 10^3 m^{-3} in October and 2116 x 10^3 m^{-3} in January (Fig. 4B.11b).

Similarly, its biomass ranged from 0.5-191 $x10^2$ m^{-3} (avg.30.5± 61.5 $x10^2$ m^{-3}) high and low values were reported in the month of January and October respectively (Fig. 4B.13b.).

Seasonally, an average values of mesozooplankton biomass during different seasons such as NEM, SIM, SWM and FIM are 66±65.5; 5.8±4.2; 40±48 and 5±2.8 ml m^{-3} respectively (Fig. 4B.12). On the whole, highest biomass was observed during NEM and least was recorded during FIM (Fig. 4B.12). Similarly, mesozooplankton abundance was highest during NEM (744 x 10^3 m^{-3}) and lowest was during SIM (72 x 10^3 m^{-3}) (Fig. 4B.14).

4B.6.1: MESOZOOPLANKTON COMPOSITION:

The mesozooplankton composition was mainly dominated by sub class copepod (70%) throughout the region (CATS location). Amongst copepods, Calanoida, accounted on an average (63%), Poiceilostomatoida (4%), Cyclopoida (2%) and Harpacticoida with <1%. While the remaining groups contributed to <10% such as Cladocera and Decapoda (6%) each, Cheatognaths (5%) and Urochordates (4) while other groups together contributed to 8% (Fig. 4B.15).

Likewise, of the total 81 mesozooplankton species identified, 66 species with 30 genera belonged to copepods. Of the Copepods identified Calanoida were more diverse (50 species; 18 genera), while Poicilostomatoida Harpacticoida, and Cyclopoida accounted to 4, 4, 1 genus respectively (see Plate IX-XI.). The most common copepod species encountered were Calanoida- (*Acartia erythrea, A. amboinsis, Acartia spinirostris, A. negligens, Paracalanus parvus P. aculeatus, Acrocalanus* spp., *Centropages orsani, C. calaninus, C. furcatus, C. tenuremis. Eucalanus pileatus, E. subcrassus E monachus, Labidocera acuta, L. minuta, L. pavo, L. pectinata;, Temora, turbinate, T. discaudata; Pseudodiaptomus serricaudata, Undinula vulgaris* and *Canthocalaus pauper*). Poiceilostomatoida- *Corycaceus catus, C. specious, Copilia mirabilis. Oncea venusta*; Cyclopoida- *Oithona plumifera, O. spinirostirs, O.rigida*; Harpacticoida- *Euterpina acutifrons* and *Microsetella rosae.* While others groups were Urochordates (*Oikopleura* sp, *Salpa*); Cladocera (*Evadne tergestina, Penilia avirostris*); Decapoda (*Lucifer hensani, Squilla* sp) and Cheatognaths (*Sagitta betoti, S.enflata*).

Seasonally, amongst the mesozooplankton distribution Copepoda were predominant throughout the season. However during NEM, stn. G1 and G2 the copepods contributed to only 39-42%, while, fish eggs were (30-40%) On an average Copepoda contributed to 64% followed by Chaetognaths (7%), Decapods (6%), Cladocera and Tunicates (2%) each. In SIM, the dominant groups were Copepoda (77%), Decapoda (10%) Chaetognaths (4%). During SWM, Copepoda (74%), Decapoda Cladocera (6%) and Urochordates (5%). While in FIM, Copepoda (67%) followed by Cladocera (10%), Cheatognaths and Urochordates contributed to (8%) each. (Fig. 4b.16).

Mesozooplankton feeding habit reveals that on an average 40% mesozooplankton community are omnivores, 25% are herbivores and carnivores each, while 10% constitutes the detritivore/suspension feeders. Seasonwise, there was a clear shift in the grazing pattern depending upon the species composition occurring throughout the year. The carnivores were the dominant forms (58%) during the NEM, during SIM and SWM the omnivores became the major grazers with 40 and 66% respectively, while during FIM, the herbivores (40%) built up (Fig. 4B.17).

4B.7. DISCUSSION

Plankton abundance and species composition in the coastal continental shelf waters, off Goa are characterized by a high degree of spatial and temporal variability (Fig. 4B.18a,e).. Monsoonal upwelling plays s a major role in the biogeochemistry of the coastal waters (Naqvi, 2000). Low temperature (21-22°C) is often observed in subsurface and bottom waters as the offshore water upwells over the shelf (Fig. 4B.18d). However the cold, nutrient rich upwelled water does not come to the surface as a low saline (33psu) layer (created due to heavy precipitation and run off) overlies (Fig. 4B.18c). Due to unique interplay of hydrographical and biogeochemical processes shelf waters turn hypoxic followed by suboxia and anoxia (Naqvi *et al.*, 2006). As low oxygen condition intensifies from July onwards, denitrification causes massive N loss. Later on in October anoxia develops often ending up being sulfidic.(Fig. 4B.18b) As the well lit shelf water column turns anoxic, presumably it causes metabolic stress on phytoplankton and zooplankton. However as the highest production in this region so far has been observed during

hypoxic/suboxic (July-August) period (Naqvi *et al.*, 2006), it appears that certain group of phytoplankton as capable of adapting to this seasonal low oxygen regime.

Seasonality in the phytoplankton composition in the Arabian Sea has been shown previously (Devassy and Goes, 1988; Sawant and Madhupratap, 1996) wherein they have concluded that the biological response is strongly coupled with atmospheric forcing. Apart from this, bloom of unidentified photosynthetic forms predominated at station G1 during the month of January 2005, which attributed to high chlorophyll *a* concentration, 107 mg m^{-3} in the surface waters. Pigment analysis confirmed by HPLC method indicated dominance of prasinoxanthin, a marker pigment for algal community belonging to class Prasinophyceae (Bhaskar *et al.*, 2011). It was speculated that its occurrence was due to the invasion of ballast water entering into the Mandov-Zuari bay. Episodic blooms of dinoflgellates are also known to occur in coastal waters of India (Karunasagar, 1992; Nayak *et al.*, 2000).While, seasonal *Trichodesmium* bloom (Devassy *et al.*, 1979) during SIM period prevail in oligotrophic conditions. At times, chlorophyll a values are as high as 128 mg m-3 when detected near to stn. G1 (data not shown). Further, large amount of NH_4^+ (up to 3.3 μM) is released into the medium (Devassy *et al.*, 1978), which leads to proliferation and succession of other planktonic organisms (Devassy *et al.*, 1979). Hence, the biogeochemistry of this unique system complements with the finding on high abundance and phytoplankton biomass during late SWM and SIM as observed in year 2005 and 2006 respectively. The seasonal pattern and inter-annual variability of the main taxa are described and discussed. In general, spatial distribution at different stations from G1 to G5 showed a decreasing trend towards the offshore stations.

Photosynthetic prokaryotes, *Synechococcus* and *Prochlorococus* are major components of oceanic ecosystems. The former one is virtually ubiquitous in marine environment of coastal and estuarine areas (Partensky *et al.*, 1999; Olson *et al.*, 1988). Size fractionated chlorophyll data reveals that pico-autotrophs (<5-0.7 μm) (*Synocococcus* and *Procholorococuc* spp.) on an average formed the major fraction of the total phytoplankton biomass and showed relatively higher biomass towards shallow station G1 as compared to stn G5(Fig. 4A,11a) . Seasonally their average abundance was high during fall intermonsoon. This is due to the fact that during this period, most of the nitrate is already consumed by the higher autotrophs (diatoms) and

mixotrophs (dinoflagellates), hence the dominance of diatoms decreases and other green flagellates and cyanobacteria predominate (Roy, 2010).

Similar to phytoplankton distribution, mesozooplankton abundance and biomass also showed higher values at near shore stations compared to offshore stations. Copepods were the predominant group throughout the season. *Paracalanus aculeatus* and *Paracalanus parvus* are the key species of the study region. However, high biomass and abundance reported mainly during FIM was mainly due to the presence of filter feeding Cladoceran swarm. Thus, the dominance of pico autotroph, *Synecococcus* probably supported thier abundance. Earlier studies have shown also that ciliates (Christaki, *et al.*, 2001; Apple *et al.*, 2011) and appendicularians (Gorsky *et al.*, 1999) preferentially feed on *Synecococcus*. To summarize the smaller cell size fraction of chlorophyll which is dominant decreases gradually towards off coast and is taken over by the larger forms of phytoplankton and seasonally pico autotrophs played a substantial role. during FIM.

Table 4B.1.Seasonal variation of phytoplankton spp composition in 2005 over the shelf, off Goa.

Total phytoplankton species in 2005				
DIATOMS	NEM	SIM	SWM	FIM
Actonoptychus undulatus	p	p	p	
Amphiphrora spp				
Asterionella japonica	p	p	p	p
Asteromphalus heptactis	p			
Bacteriastrum furcatum	p	p		
Bacteriastrum hyalinum			p	
Bacteriastrum spp		p	p	
Bellerochea horologicalis	p		p	
Cerataulina spp	p		p	p
Cerataulina spp.				
Chaetoceros affinis			p	
Chaetoceros curvisetus	p	p	p	p
Chaetoceros danicus	p			
Chaetoceros decipiens		p		
Chaetoceros loranzianus		p	p	p
Chaetoceros spp.	p	p	p	p
Climacodinium frauenfeldianum	p			
Climacospenia elongata	p			
Coconeis				
Coconeis spp.	p	p		p
Coconeis spp.				
Corethron hystrix	p	p	p	
Coscindiscus marginatus	p	p	p	p
Coscinodiscus granii	p			
Coscinodiscus lineatus	p		p	
Coscinodiscus radiatus		p	p	
Coscinodiscus spp.	p	p	p	p
Diploneis weissflogii	p			
Ditylum brightwelli	p	p	p	p
Ditylum sol	p	p	p	
Eucampia cornuta		p	p	p
Fragilaria oceanica	p			p
Fragilaria spp	p		p	
Guinardia spp	p	p		
Guinardia striata		p	p	p
Gyrosigma balticum			p	

Hemiaulus hauckii		p		
Hemiaulus sinensis			p	
Hemiaulus spp.		p	p	
Lauderia spp.	p	p	p	
Leptocylindrus danicus	p	p	p	
Leptocylindrus minimus	p	p		p
Melosira spp	p	p		
Navicula transitans var.derasa	p	p	p	p
Navicula transitans var.derasa f.delicatula	p	p	p	p
Navicula distans	p			
Navicula spp	p	p	p	P
Navicula vanhoeffenii	p			
Nitzschia spp.	p	p	p	P
Nitzschia closterium	p	p	p	P
Nitzschia longissima	p	p	p	
Nitzschia sigma			p	P
Odontella mobiliensis	p	p	p	
Odontella sinensis	p	p	p	P
Planktoniella sol		p	p	P
Pleurosigma angulatum		p		
Pleurosigma directum	p			P
Pleurosigma elongatum	p		p	P
Pleurosigma spp.				
Pleurosigma spp.	p			
Pseudonitzschia seriata		p	p	P
Pseudonitzschia spp				
Rhaphoneis setigera	p	p	p	P
Rhizosolenia alata	p		p	
Rhizosolenia hebetata			p	
Rhizosolenia robusta	p			P
Rhizosolenia setigera				
Rhizosolenia spp.		p	p	P
Rhizosolenia stolterfothii		p	p	
Rhizosolenia styliformis	p	p		
Schroederella sp.	p			
Skeletonema costatum	p	p	p	P
Stephanophysis palmeriana		p		
Streptotheca tamensis	p	p	p	

Thalassionema nitzschoides	p	p	p	p
Thalassiosera subtilis	p	p	p	p
Thalassiothrix fauenfeldii			p	
Thalassiothrix spp	p	p		
DINOFLAGELLATES				
Alexandrium spp	p			
Amphidinium spp.	p	p		p
Ceratium furca	p	p	p	p
Ceratium fusus			p	p
Ceratium kofoidii	p			p
Ceratium spp.				p
Ceratium horridum		p		
Ceratium tripos				p
Cochlodinium sp	p			p
Dinophysis acuminata	p	p	p	p
Dinophysis caudata				p
Dinophysis dens			p	
Gymnodinium spp	p	p	p	p
Gyrodinium spp.	p	p		p
Heterocapsa spp.	p			p
Ornithocercus thumii				p
Oxytoxum spp.	p			
Polykrikos sp.	p			
Prorocentrum dentatum	p	p	p	p
Prorocentrum gracile	p	p		p
Prorocentrum micans	p	p	p	p
Protoentrum minimum	p			
Protoperidinium oceanicum				p
Protoperidinium spp.	p	p	p	p
Protoperidinium steinii	p	p	p	p
Protoperidiniun depressum	p			p
Pyrophacus steinii	p	p	p	p
Scrippsiella spp.		p	p	
Scrippsiella trochoidea	p	p		
SILICOFLAGELLATES				
Dictyocha fibula	p	p	p	p
Ebria tripartita	p			
Prasinophycea				
prasinophyte bloom	p			

Table: 4B.2. Seasonal variation of phytoplankton species composition in 2006 over the shelf, off Goa.

Total phytoplankton species in 2006				
DIATOMS	NEM	SIM	SWM	FIM
Actonoptychus undulatus		p	p	p
Amphiphrora spp	p	p		
Asterionella japonica	p		p	p
Asteromphalus spp	p			
Bacillaria paradoxa	p			
Bacteriastrum hyalinum	p	p		
Bellerochea malleus	p	p		
Cerataulina spp				p
Chaetoceros affinis	p	p		
Chaetoceros coarctatus	p			
Chaetoceros curvisetus	p	p	p	p
Chaetoceros danicus	p			
Chaetoceros decipens	p		p	
Chaetoceros loranzianus	p	p	p	p
Chaetoceros spp.	p	p	p	p
Climacodinium frauenfeldianum	p		p	p
Coconeis spp.	p	p	p	p
Coscinodiscus eccentricus			p	
Coscinodiscus spp.	p	p	p	p
Cosinidiscus lineatum	p			
Cosinodiscus granii				p
Cosinodiscus marginatus	p	p	p	p
Cosinodiscus radiatus	p	p	p	p
Cyclotella spp	p		p	p
Dactyliosolen spp			p	p
Diploneis spp	p	p		
Ditylum brightwelli	p	p	p	p
Ditylum sol	p	p	p	p
Eucampia cornuta	p	p		p
Eucampia zodiacus	p			p
Guinardia delicatula	p	p		p
Guinardia flaccida	p	p		p
Guinardia striata	p	p	p	p

Hemiaulus spp.	p	p	p	p
Lauderia annulata	p	p	p	p
Lauderia spp	p			
Leptocylindrus danicus	p	p	p	p
Leptpcylindrus minimus	p	p	p	p
Melosira spp	p	p		p
Melosira sulcata	p	p	p	p
Navicula transitans var.derasa	p	p	p	p
Navicula transitans var.derasa f.delicatula	p	p	p	p
Navicula septentrionalis			p	p
Navicula spp.	p	p	p	p
Navicula vanhoeffenii			p	
Nitzschia longissima				p
Nitzschia spp.	p	p		p
Nitzschia closterium	p	p		
Odontella mobiliensis	p	p	p	
Odontella sinensis	p	p	p	p
Planktoniella sol	p	p	p	p
Pleurosigma aesturii	p	p		
Pleurosigma angulatum	p	p	p	p
Pleurosigma balticum		p	p	p
Pleurosigma directum	p		p	p
Pleurosigma elongatum			p	p
Pleurosigma normanii	p	p	p	p
Pleurosigma spp.	p	p	p	p
Pseudonitszchia serieta				p
Pseudonitszchia spp	p	p	p	p
Rhizosolenia alata		p	p	
Rhizosolenia calcar-avis	p			
Rhizosolenia crassipina	p		p	
Rhizosolenia hebatata		p		
Rhizosolenia imbricata		p	p	p
Rhizosolenia robusta	p	p		
Rhizosolenia setigera	p	p	p	p
Rhizosolenia spp.	p	p	p	p
Rhizosolenia stolterfothii				p
Rhizosolenia syliformis	p	p	p	p
Schroederella spp	p	p	p	
Skeletonema costatum	p	p	p	p

Streptotheca tamensis	p			
Surrella spp	p	p		
Stephanophysis sp			p	
Synedra spp	p			p
Thalasiothix fauenfeldii	p	p	p	p
Thalassiothix spp			p	p
Thalassionema nitzchoides	p	p	p	p
Thalassiosera spp	p	p	p	p
Thalassiosera subtilus	p	p	p	p
Triceratium spp	p	p		
DINOFLAGELLATES				
Amphidinium spp.	p	p	p	p
Ceratium concilians		p		
Ceratium furca	p	p	p	p
Ceratium fusus	p	p	p	p
Ceratium kofoidii	p			
Ceratium lineatum	p			
Ceratium macroceros	p			
Ceratium trichoceros	p			
Ceratium tripos	p			
Cochlodinium catenatum	p			p
Dinophysis miles	p	p		
Dinophysis acuminata	p		p	
Dinophysis caudata	p	p	p	
Gonyaulax spp		p	p	p
Gymnodium spp.	p	p	p	p
Gyrodinium spp	p	p	p	p
Heterocapsa spp	p		p	p
Noctiluca spp			p	
Ornithorincus thumii	p			
Phalaroma rotundatum			p	
Podolampus spp.				p
Podolampus palmipes		p		
Polykrikos spp		p	p	p
Prorocentrum dantatum	p	p	p	
Prorocentrum gracile	p	p	p	p
Prorocentrum micans	p	p	p	p
Protoperidinium conicum		p	p	
Protoperidinium depressum	p	p	p	p

Protoperidinium elegans		p		
Protoperidinium pentagonum	p	p	p	p
Protoperidinium spp.	p	p	p	p
Protoperidinium steinii	p	p	p	p
Pyrophacus steinii	p	p	p	p
Scrippsiella spp.	p	p	p	p
SILICOFLAGELLATE				
Dictyocha fibula	p	p		
Dictyocha octactis		p		
BLUE GREEN ALGAE				
Trichodesmium erythreum		p		
Prasinophycea				
Phaeocystis globosa				p

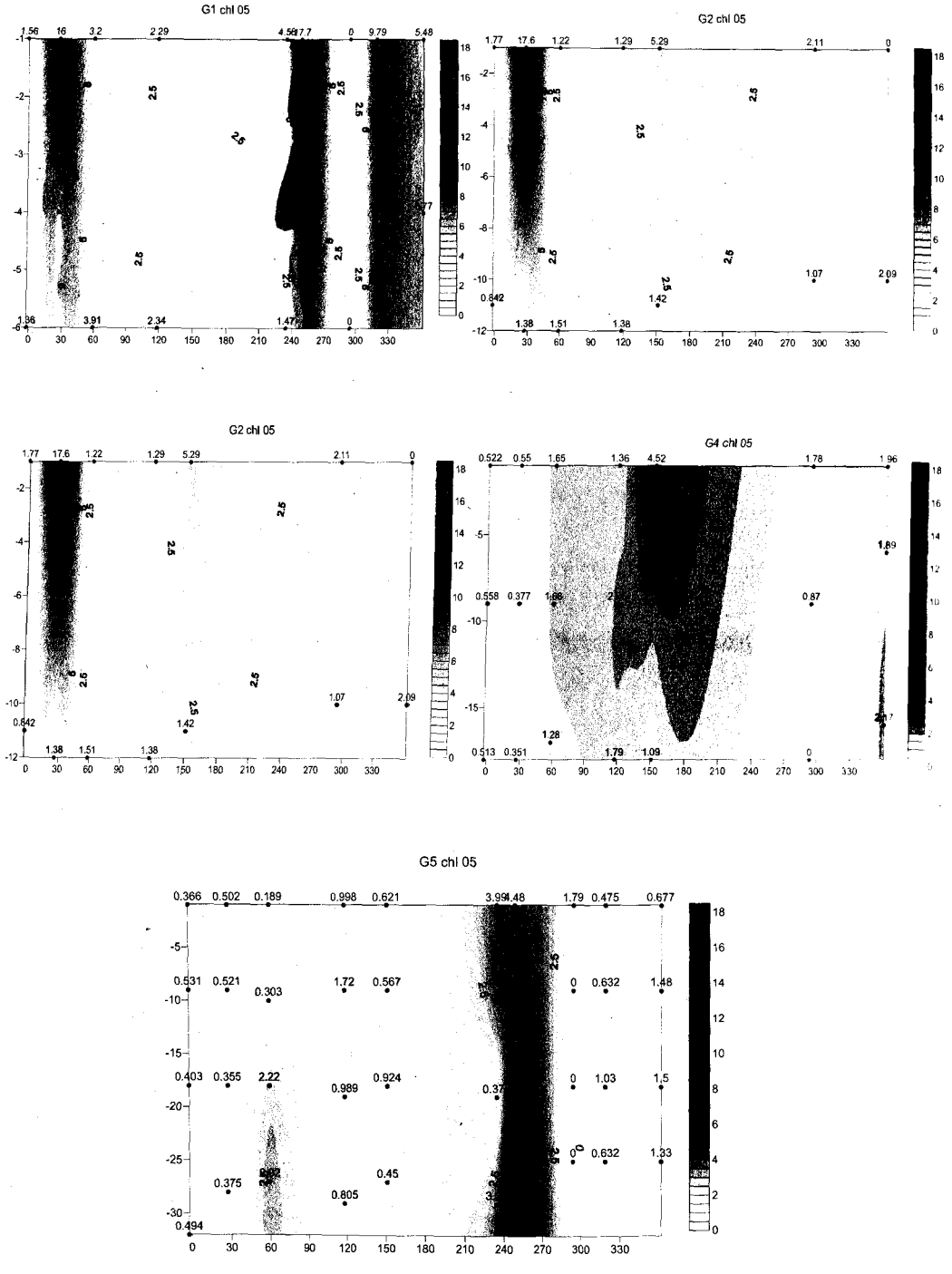


Fig. 4B.1 Annual variations of chlorophyll a (mg m^{-3}) at the CaTS coastal transect (stn G1-G5) during 2005.

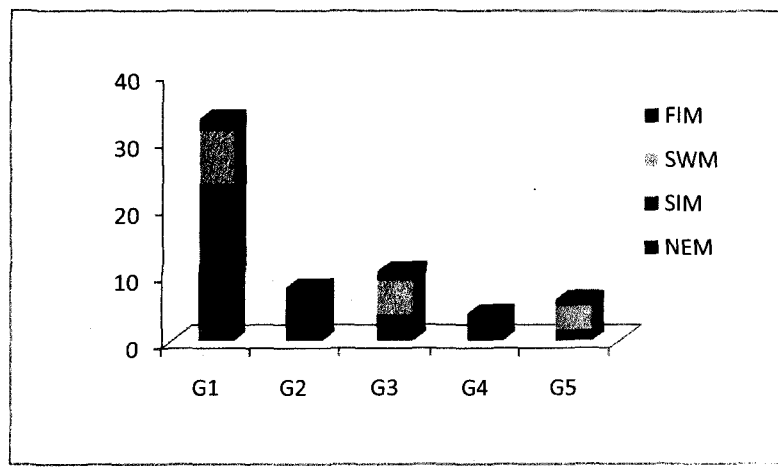


Fig. 4B.2a. Spatial variation of chlorophyll *a* at different CATS location during the year 2005.

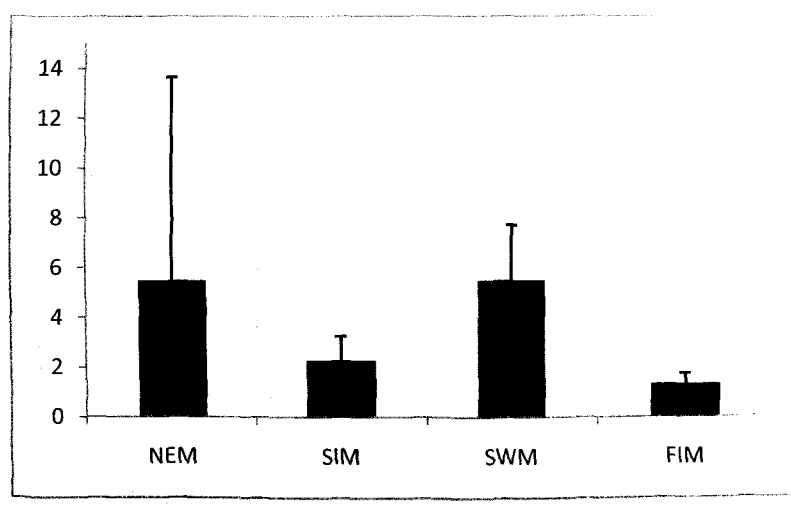


Fig. 4B.2b. Seasonal variation of chlorophyll *a* at different CATS location during the year 2005.

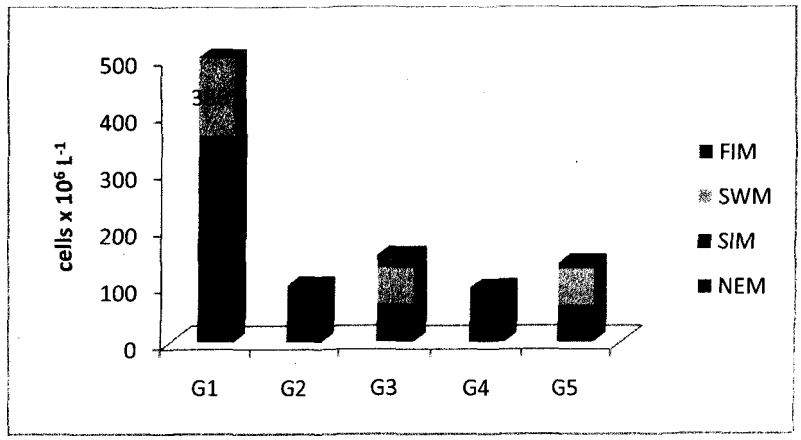


Fig. 4B.3a. Spatial and temporal variation of phytoplankton abundance at different CATS location.

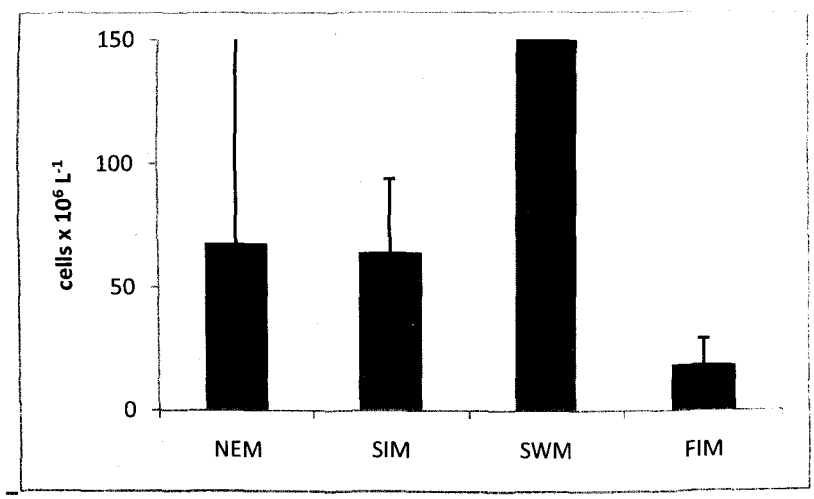


Fig. 4B.3b. Seasonal variation of phytoplankton cell abundance in the year 2006.

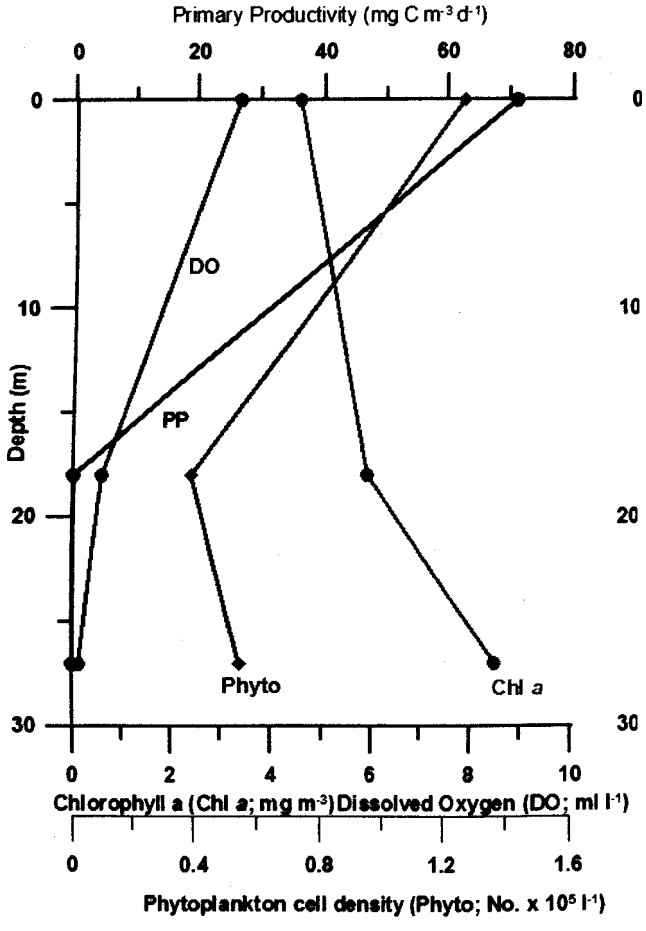


Fig. 4B.4. Vertical profile of primary productivity (pp), chlorophyll a (chla), phytoplankton abundance (phyto) and dissolved oxygen at the CaTS site during September 2006.

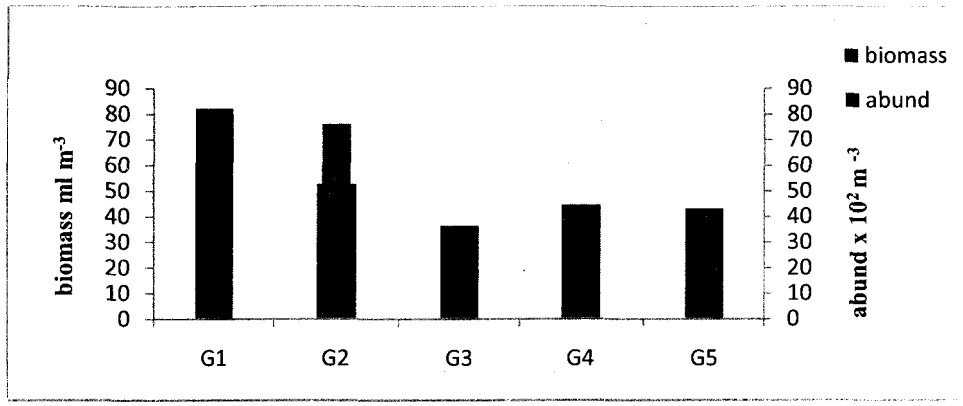


Fig. 4B.5a. Spatial annual variation mesozooplankton biomass and abundance at different CaTS locations collected during Dec'04 to Nov'05.

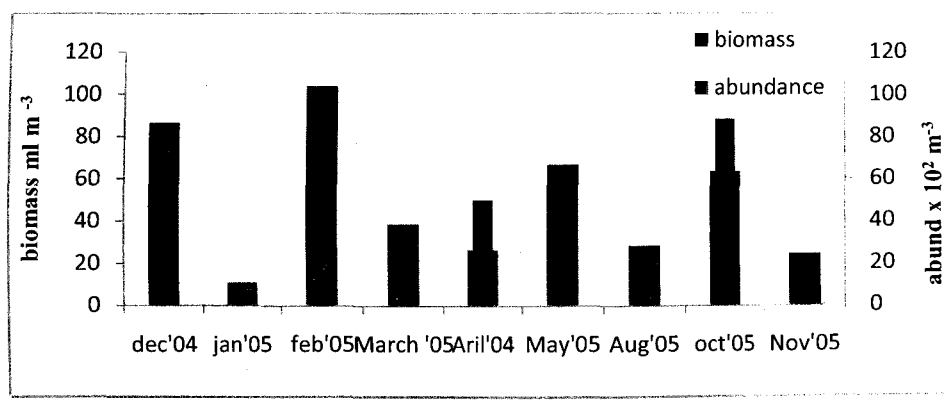


Fig. 4B.5b. Monthly variation of mesozooplankton biomass and abundance at different CATS locations during Dec'04 to Nov'05

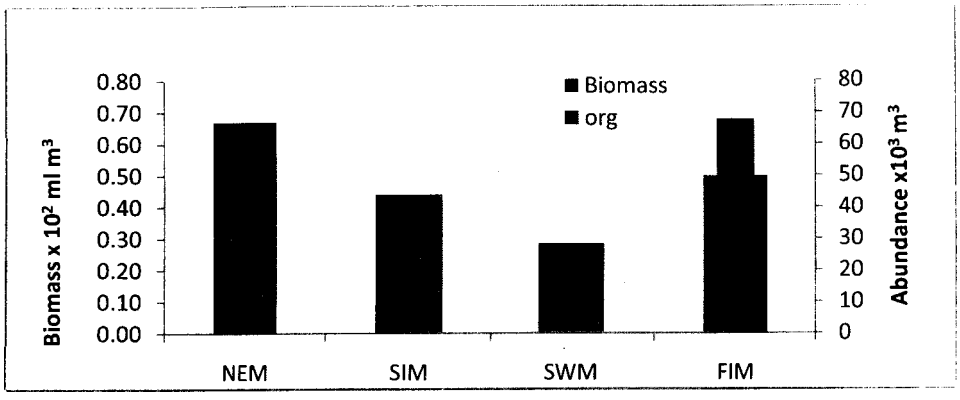


Fig. 4B.6. Seasonal variation of mesozooplankton biomass and abundance at different CATS locations during Dec'04 to Nov'05.

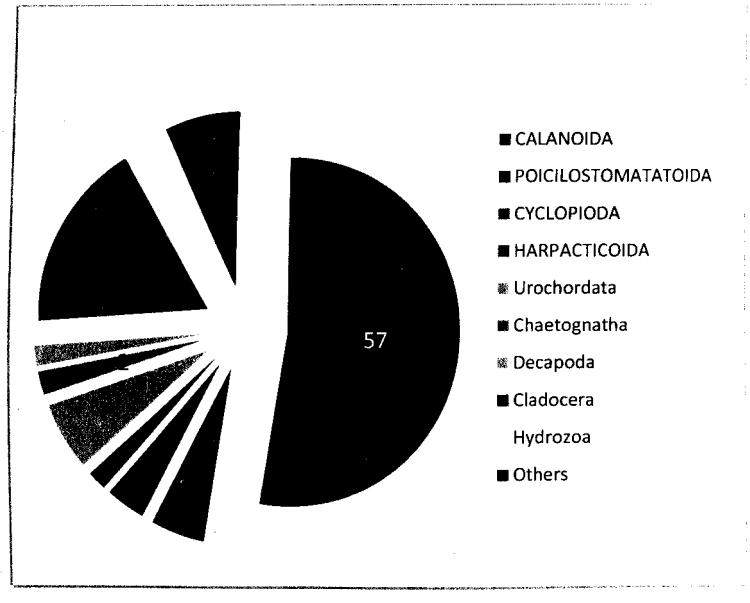


Fig. 4B.7. Average percentage distribution of mesozooplankton composition at CaTS location.

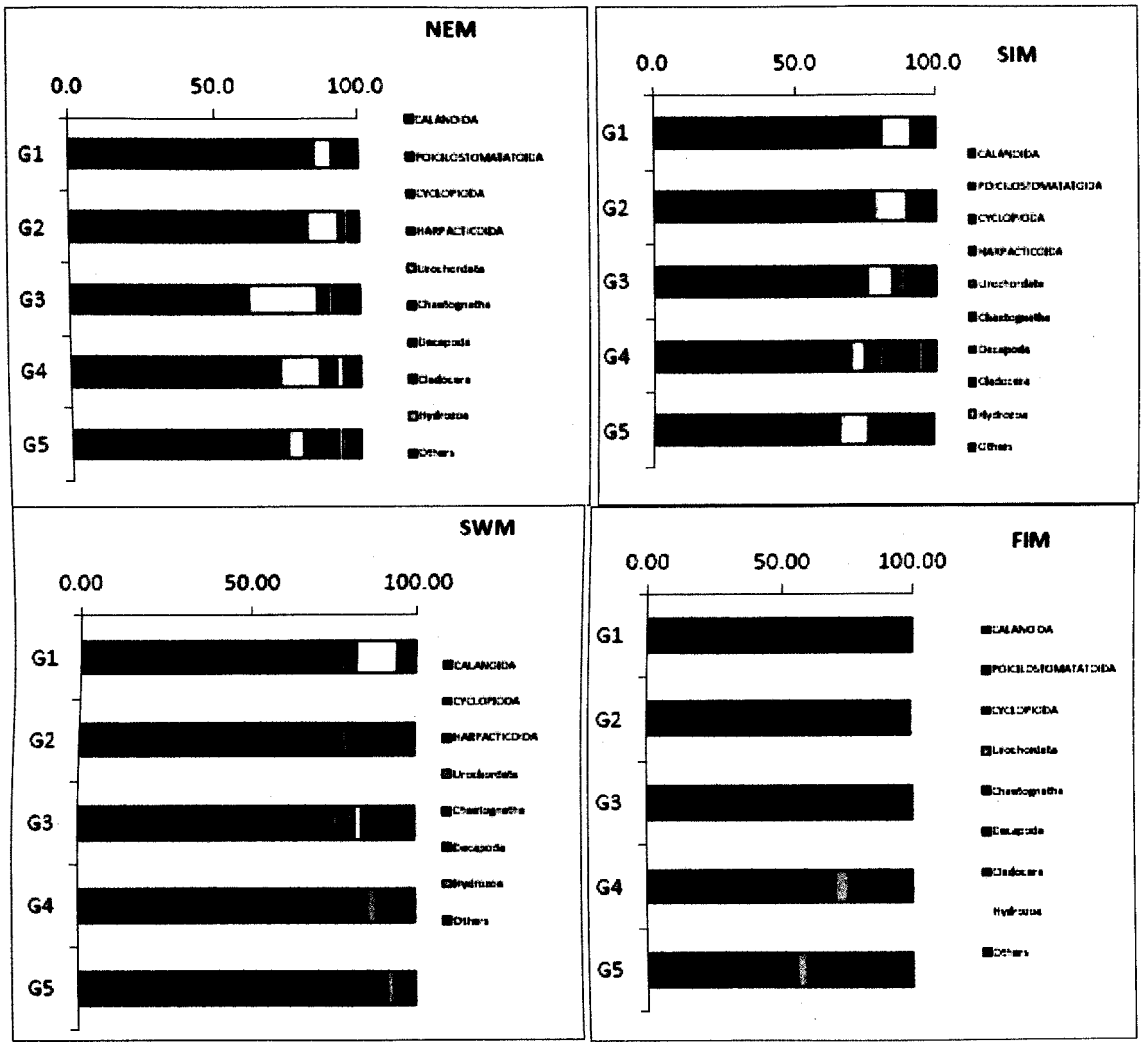


Fig. 4B.8. Average seasonal representation of major groups in mesozooplankton community at CATS location during different seasons in 2005.

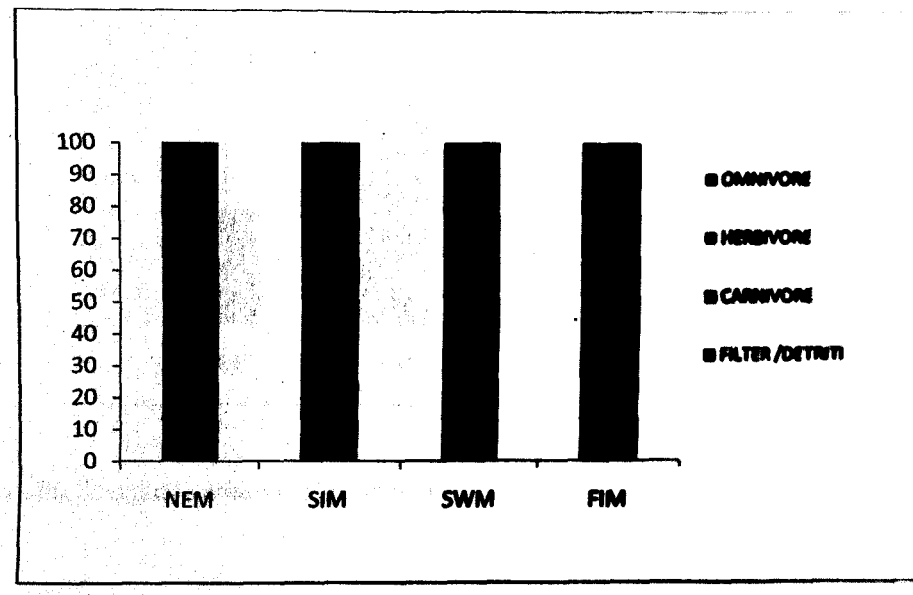


Fig. 4B.9. Seasonal mean percentage distribution of mesozooplankton community based on their feeding habits at CATS location.

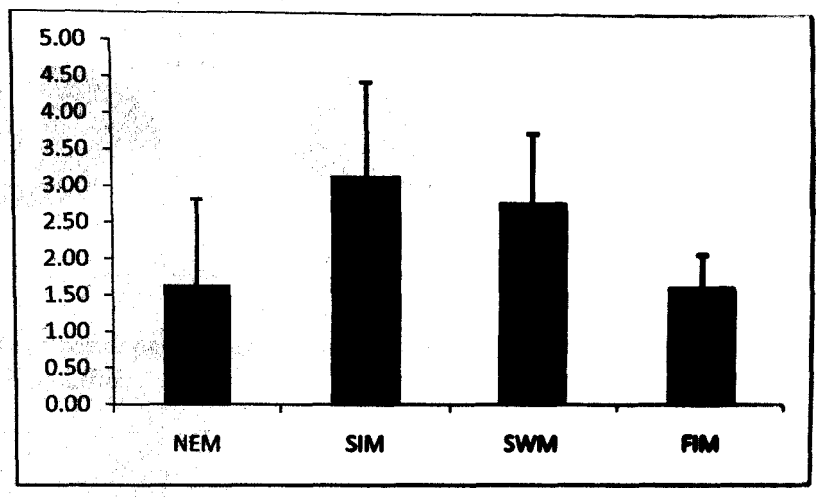


Fig. 4B.10a. Seasonal variation of phytoplankton biomass (chlorophyll *a*) in the year 2006

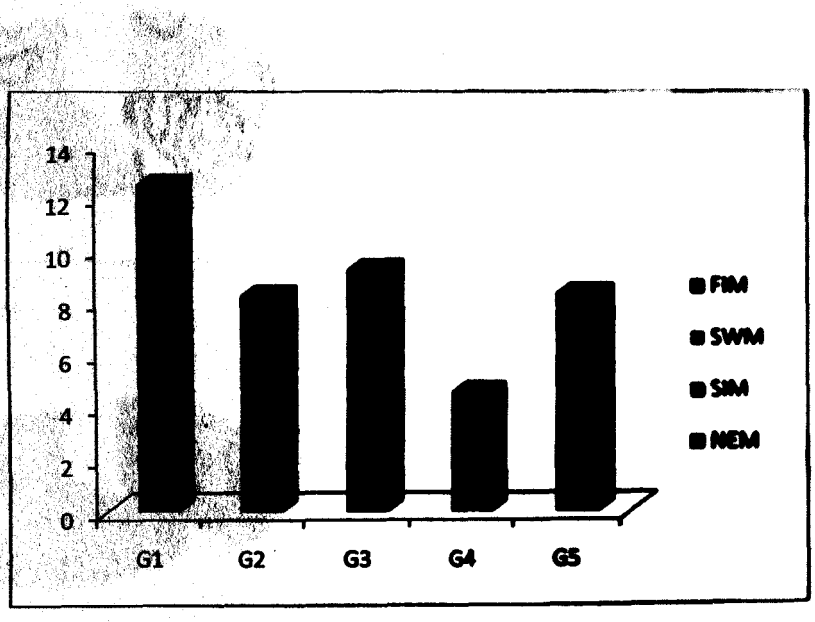


Fig. 4B.10b. Spatial and temporal variation of phytoplankton biomass (chlorophyll *a*) at different CaTS location during 2006.

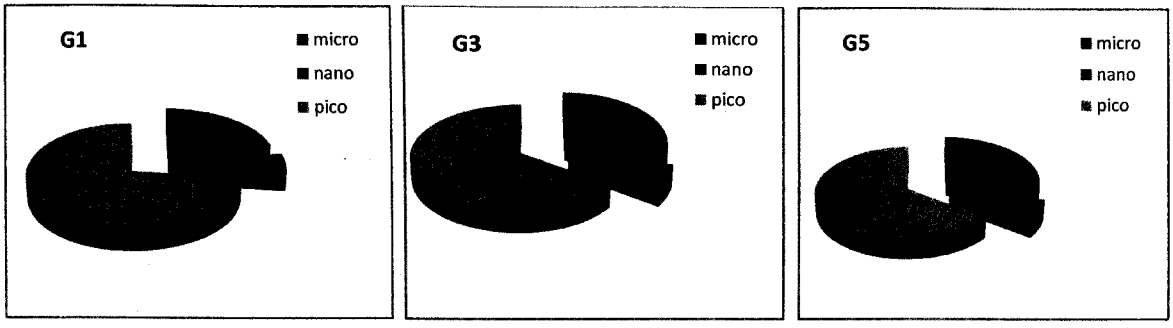


Fig. 4B.11a. Spatial percentage distribution ofsize fractionated chlorophylla at different CATS location during 2006.

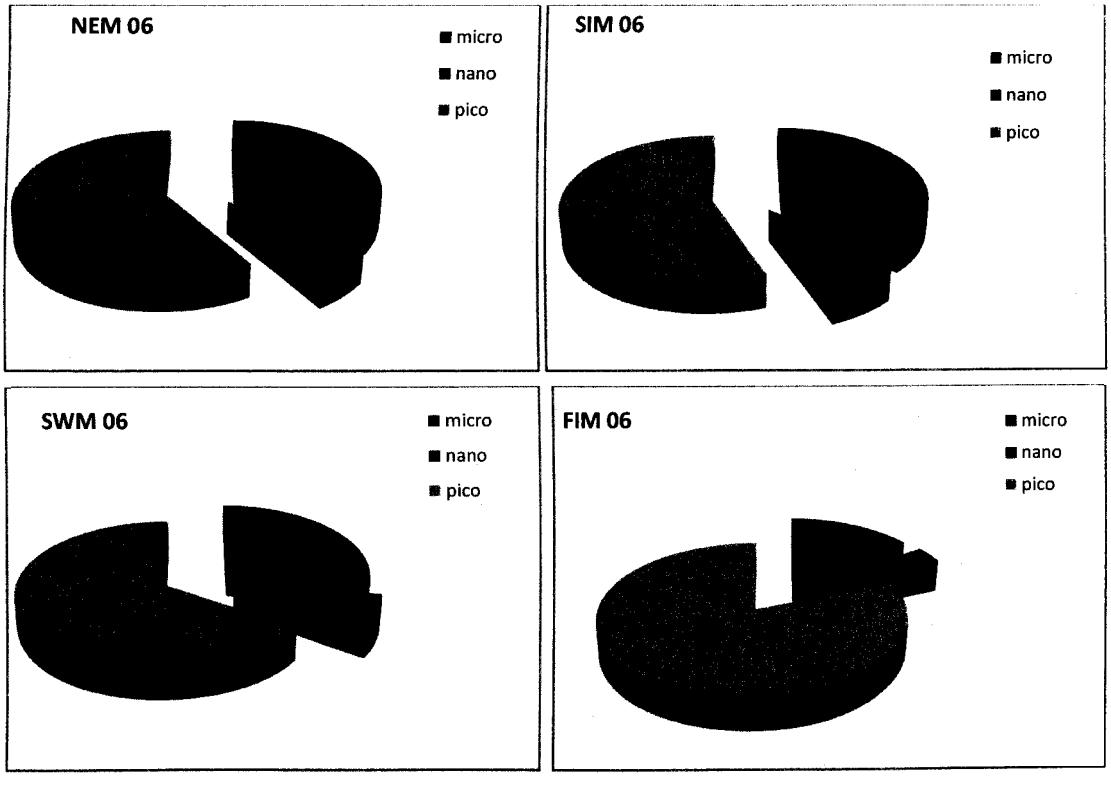


Fig. 4B.11b. Average percentage distribution of size fractionated chlorophylla in different seasons at CATS location during 2006.

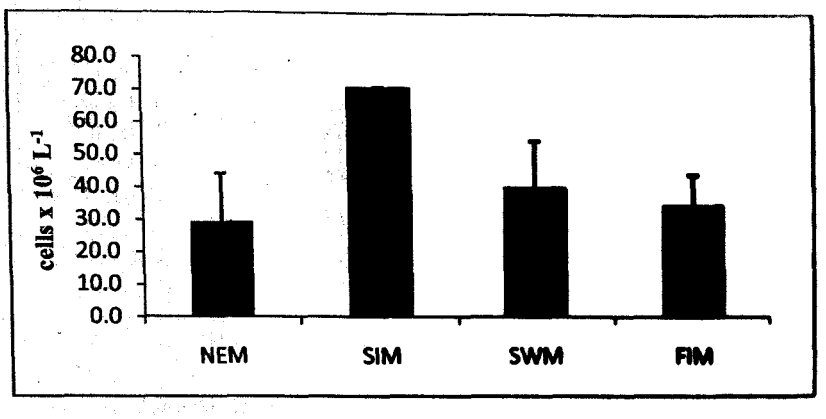


Fig. 4B.12a. Seasonal variation of phytoplankton cell abundance in the year 2006.

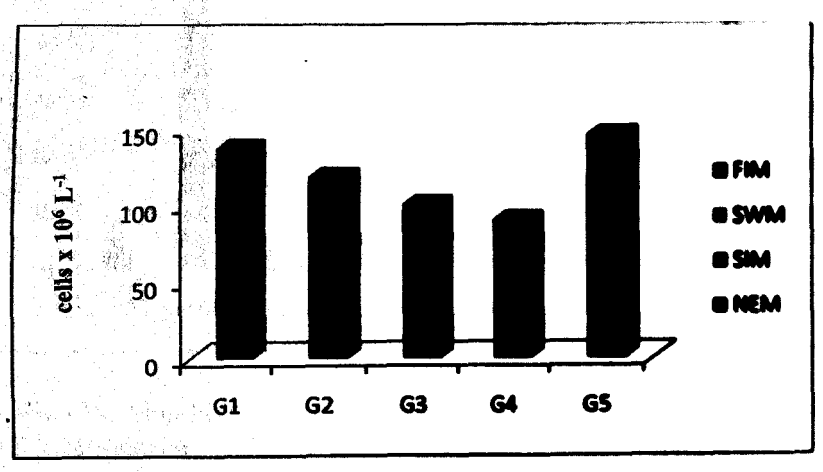


Fig. 4B.12b. Spatial and temporal variation of phytoplankton abundance at different CATS location.

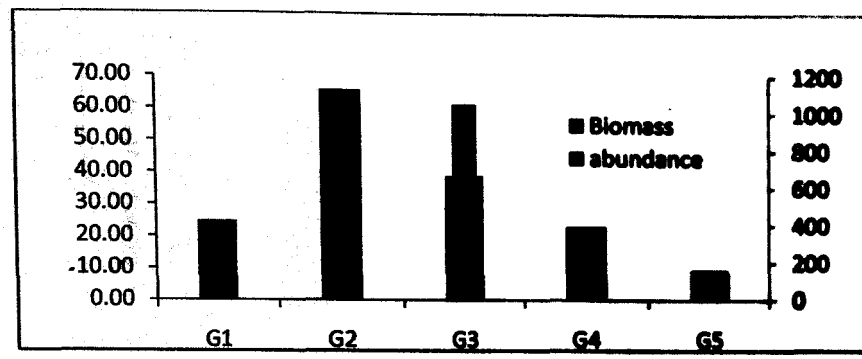


Fig. 4B.13a. Spatial annual variation zooplankton biomass and abundance at different CATS locations collected during Dec'05 to Nov'06.

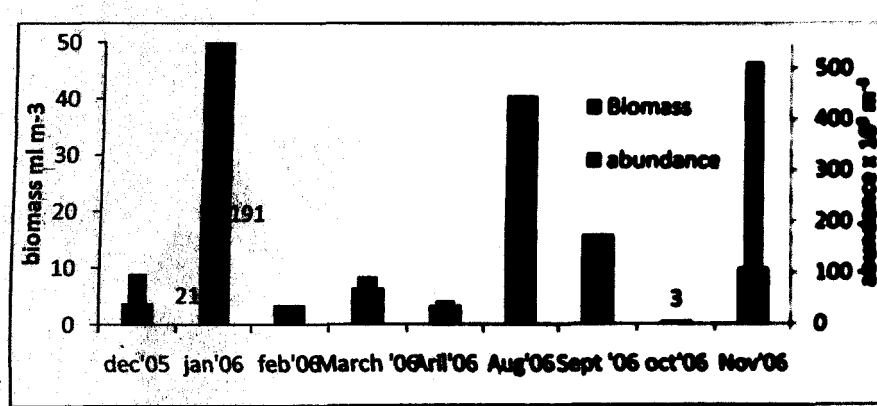


Fig. 4B.13b. Monthly variation of mesozooplankton biomass and abundance at different CATS locations during Dec'05 to Nov'06.

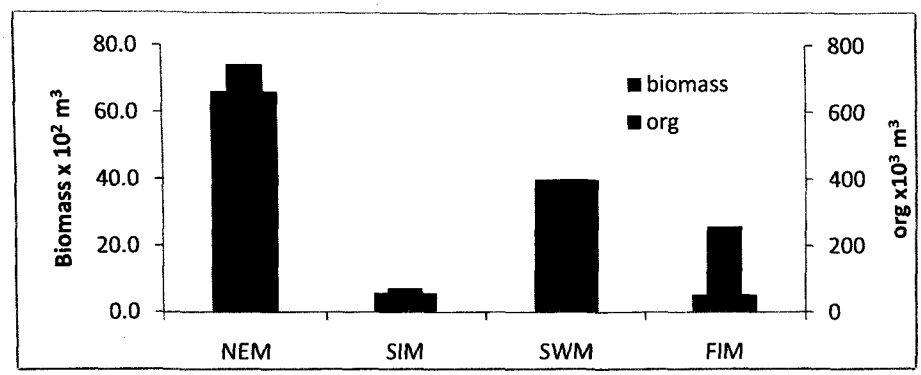


Fig. 4B.14. Seasonal variation of mesozooplankton biomass and abundance during Dec'05 to Nov'06.

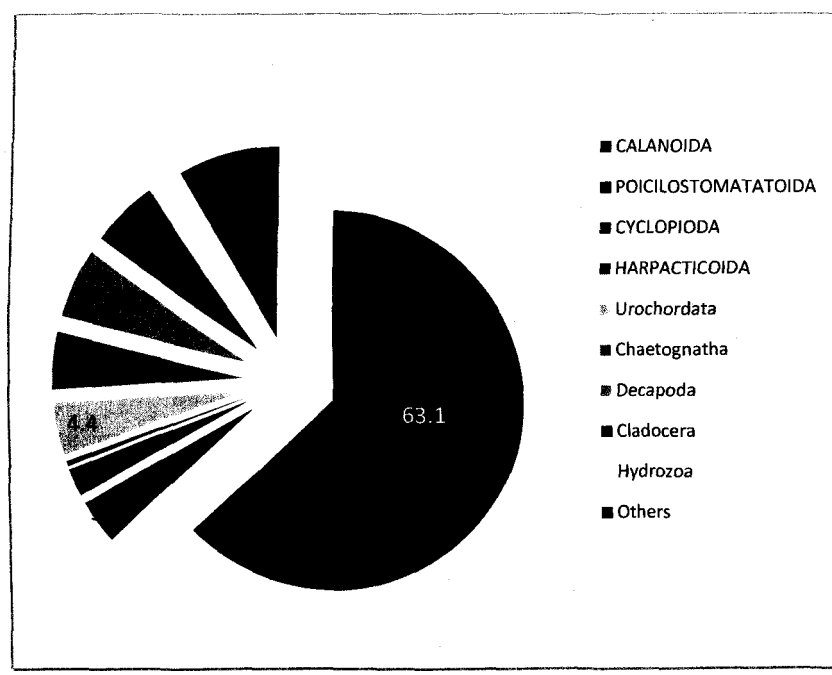


Fig. 4B.15. Average percentage distribution of mesozooplankton composition at CATS location.

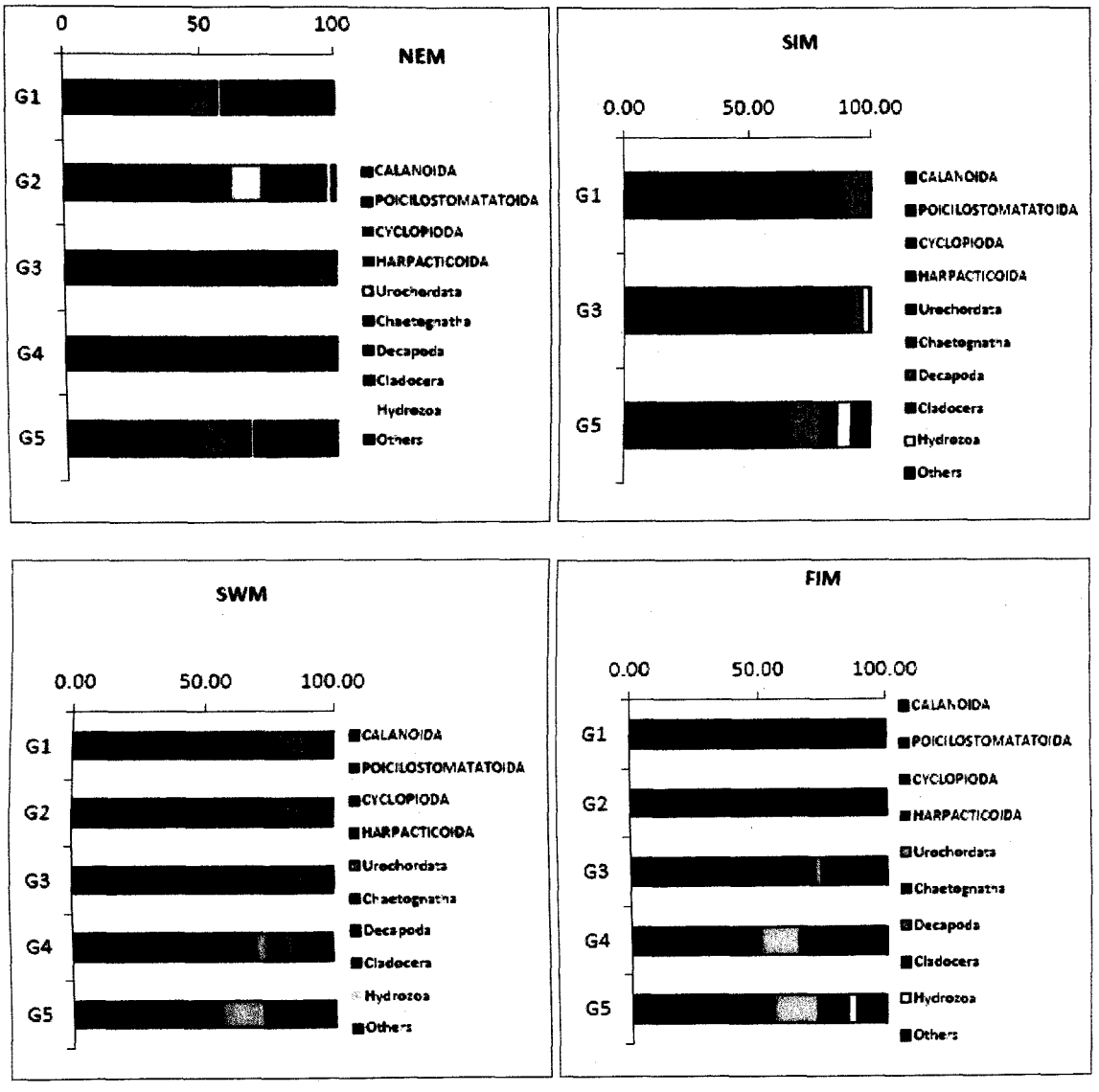


Fig. 4B.16. Average seasonal representation of major groups in mesozooplankton community at CATS location

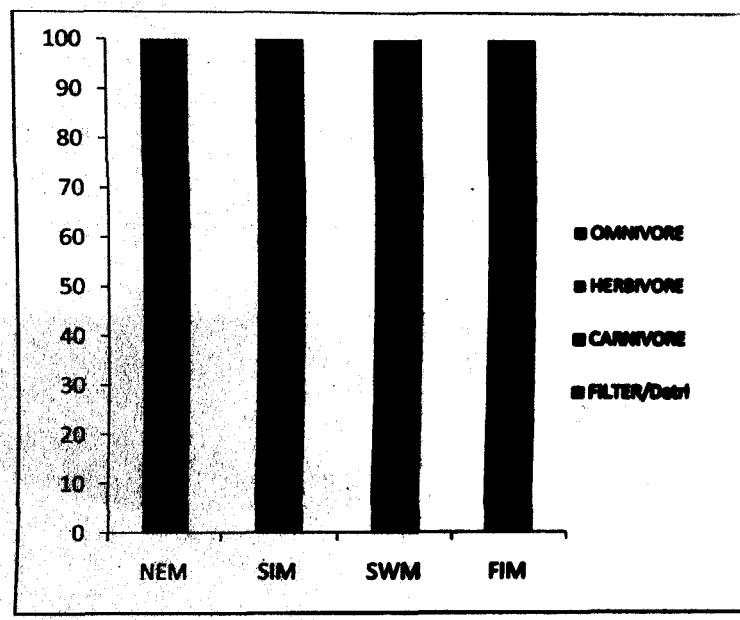


Fig. 4B.17. Seasonal mean percentage distribution of mesozooplankton community based on their feeding habits at CATS location in 2006.

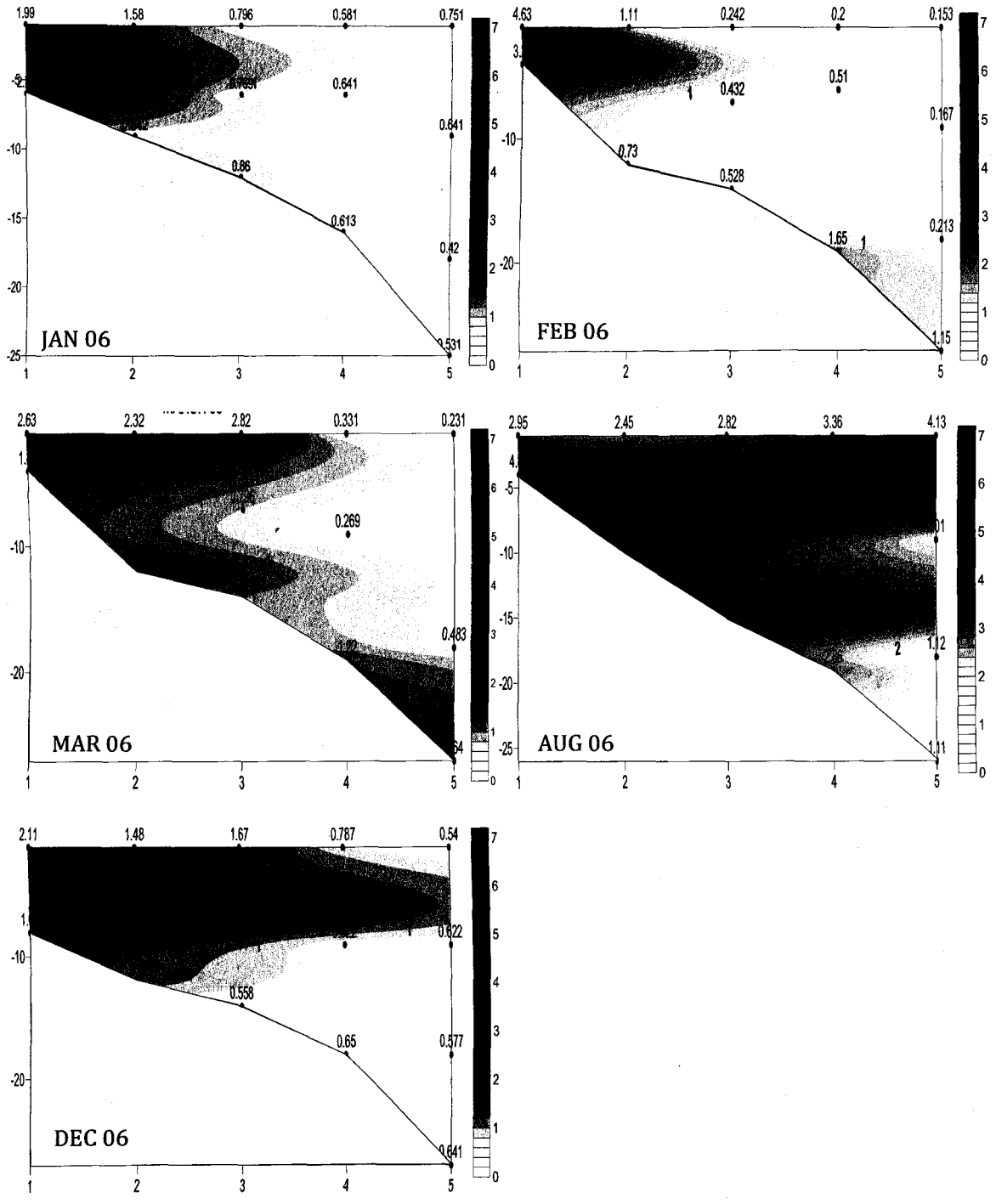


Fig. 4B.18a. Spatial and temporal variation of Chlorophyll *a* (mg m^{-3}) over the shelf off Goa during 2006. X-axis denotes distance (Km) from the coast and Y-axis denotes depth (m).

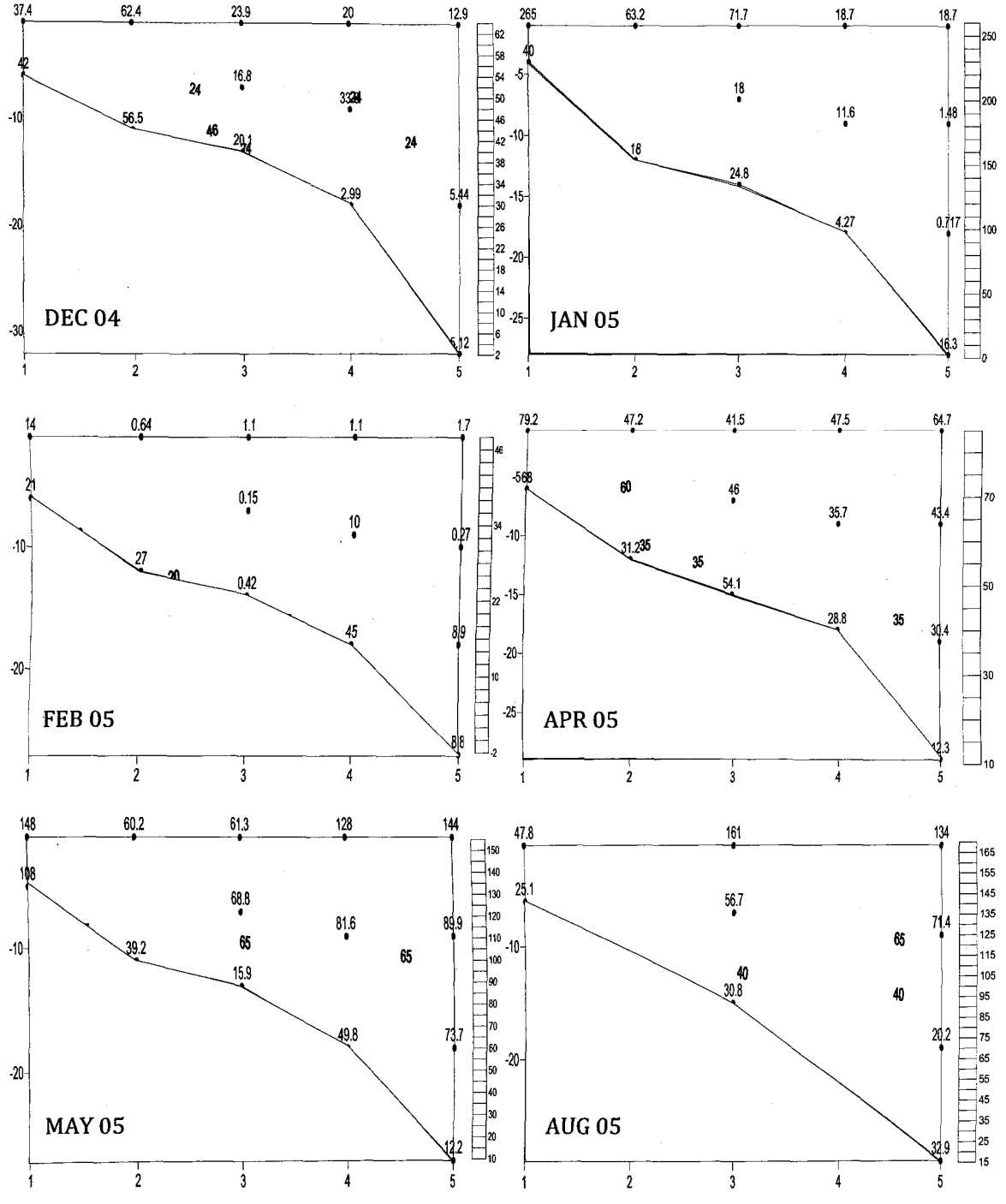


Fig. 4B.18b. Spatial and temporal variation of phytoplankton abundance (10^6 cells/L) over the shelf off Goa during 2004-2006. X-axis denotes distance (Km) from the coast and Y-axis denotes depth (m).

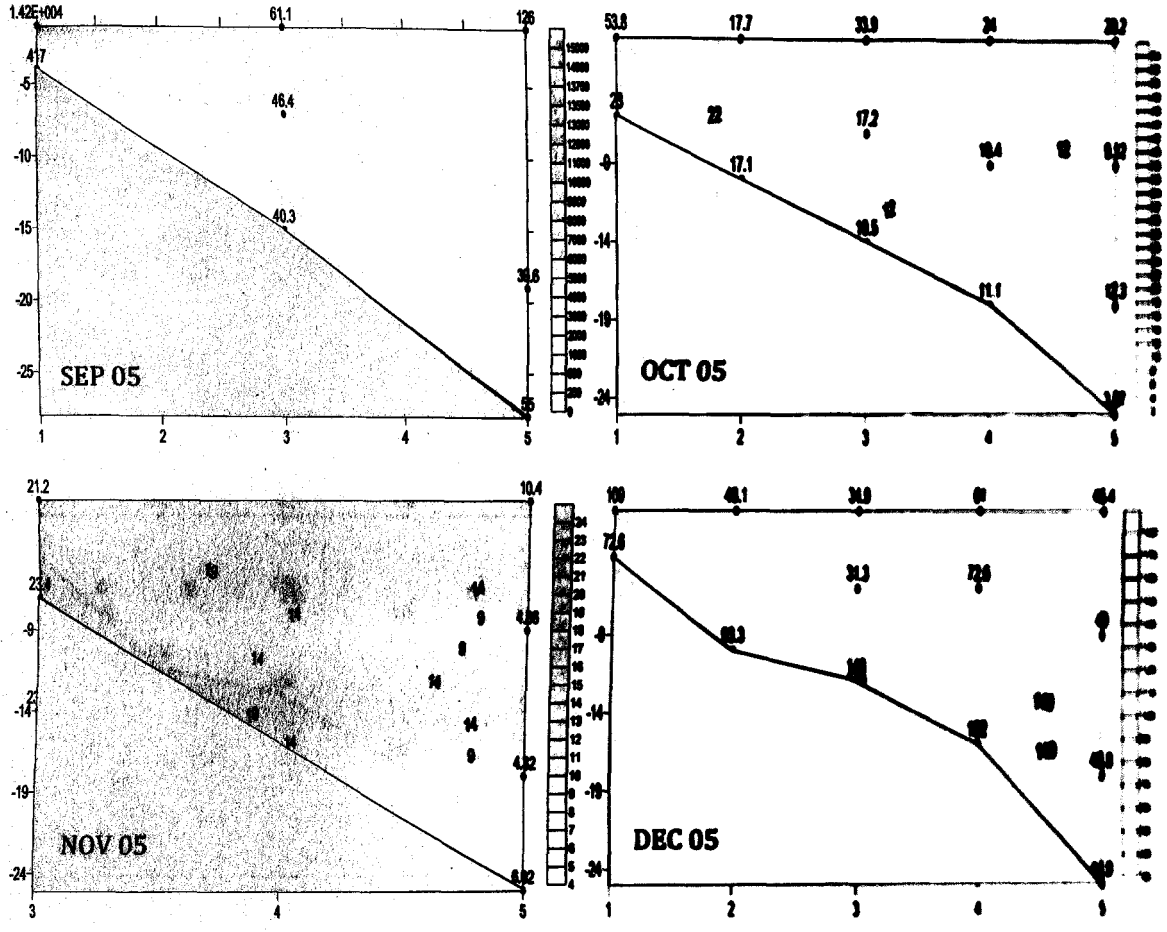


Fig. 4B.18b. Spatial and temporal variation of phytoplankton abundance (10^6 cells/L) over the shelf off Goa during 2004-2006. X-axis denotes distance (Km) from the coast and Y-axis denotes depth (m).

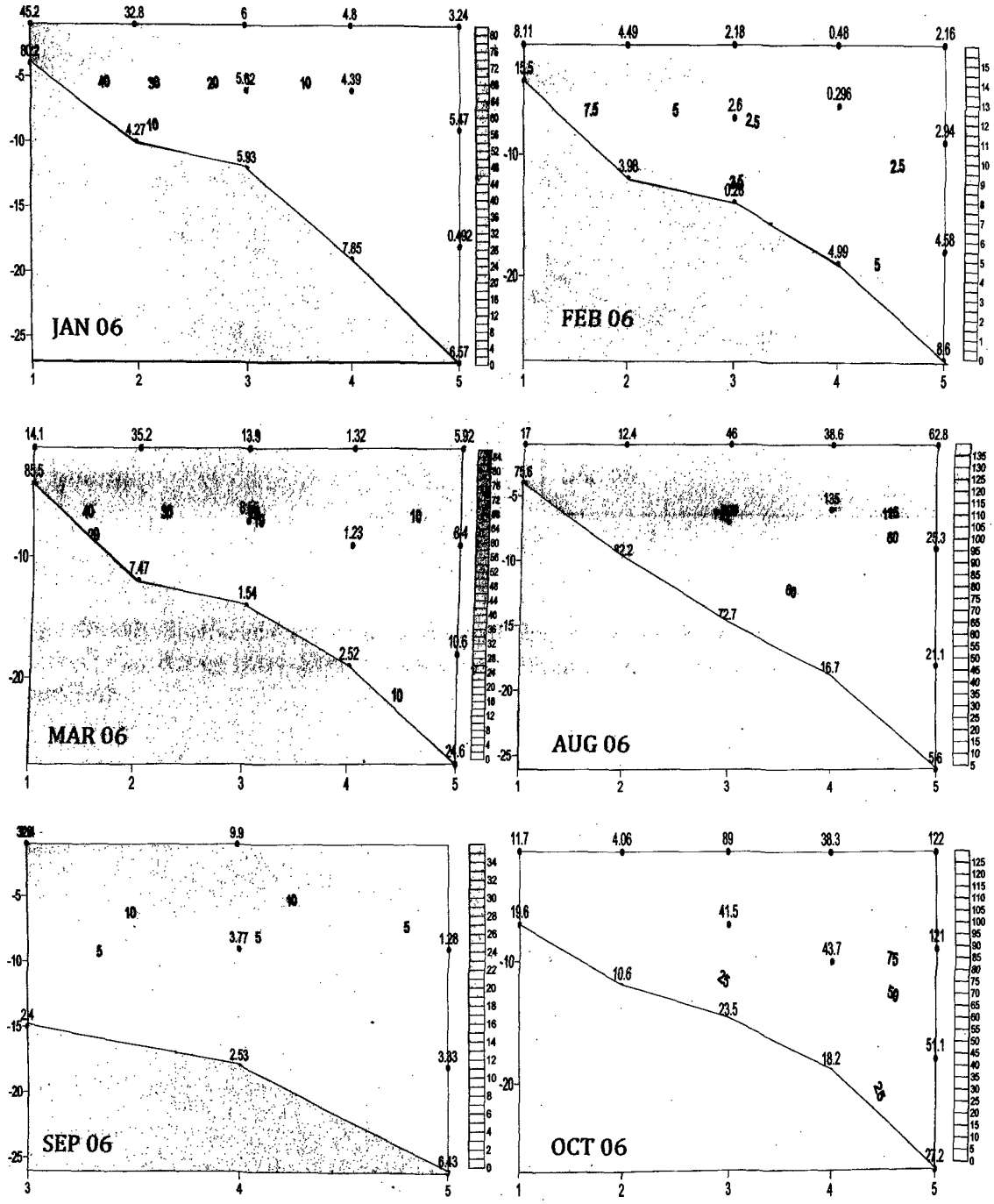


Fig. 4B.18b. Spatial and temporal variation of phytoplankton abundance (10^6 cells/L) over the shelf off Goa during 2004-2006. X-axis denotes distance (Km) from the coast and Y-axis denotes depth (m).

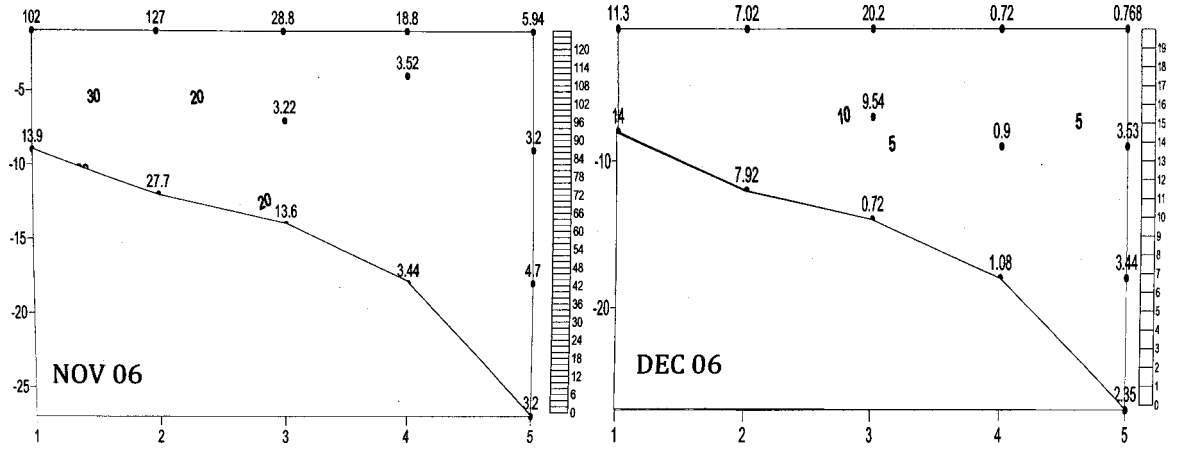


Fig. 4B.18b. Spatial and temporal variation of phytoplankton abundance (10^6 cells/L) over the shelf off Goa during 2004-2006. X-axis denotes distance (Km) from the coast and Y-axis denotes depth (m).

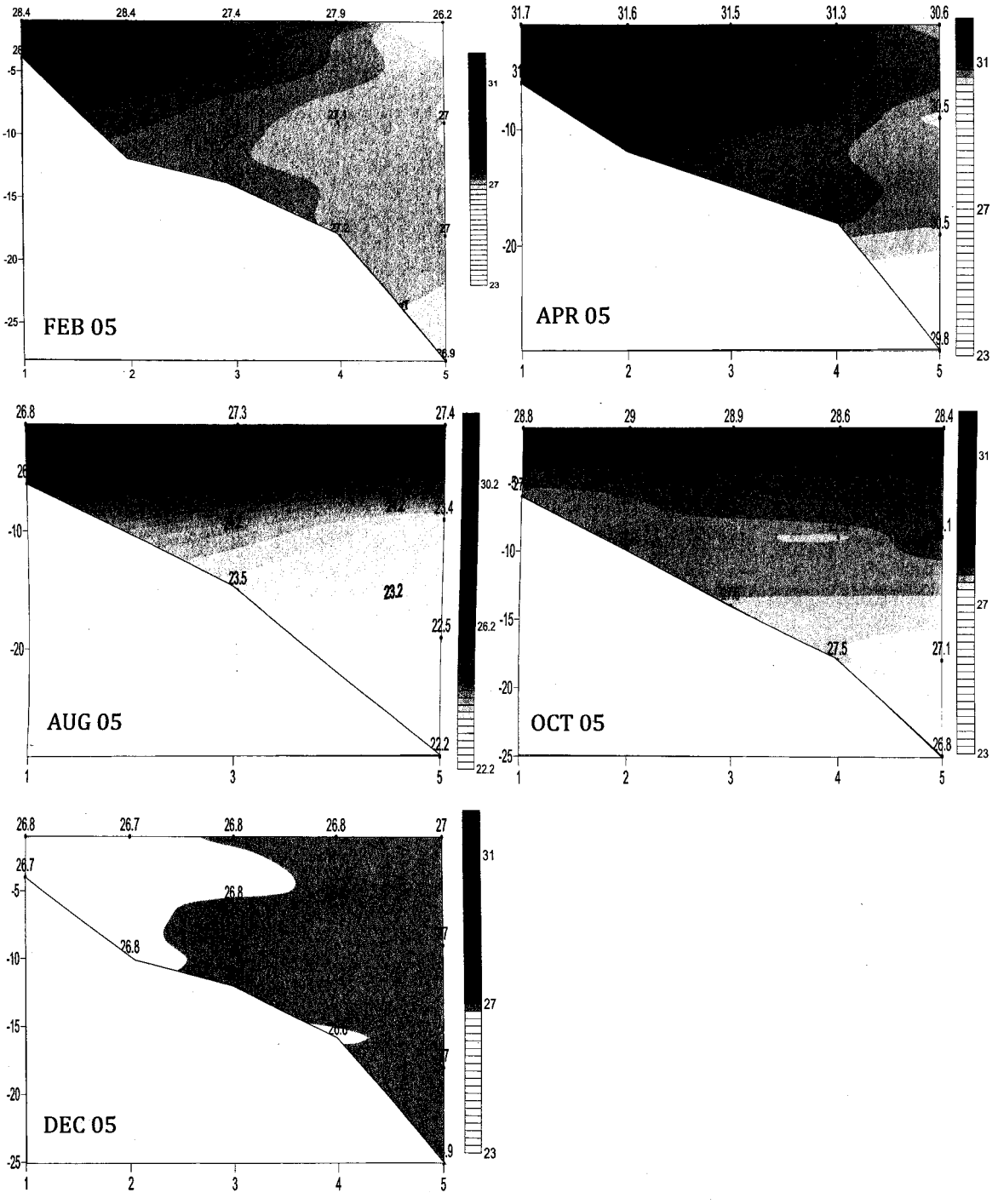


Fig. 4B.18c. Spatial and temporal variation of temperature (◦C) over the shelf off Goa during 2005-2006. X-axis denotes distance (Km) from the coast and Y-axis denotes depth (m).

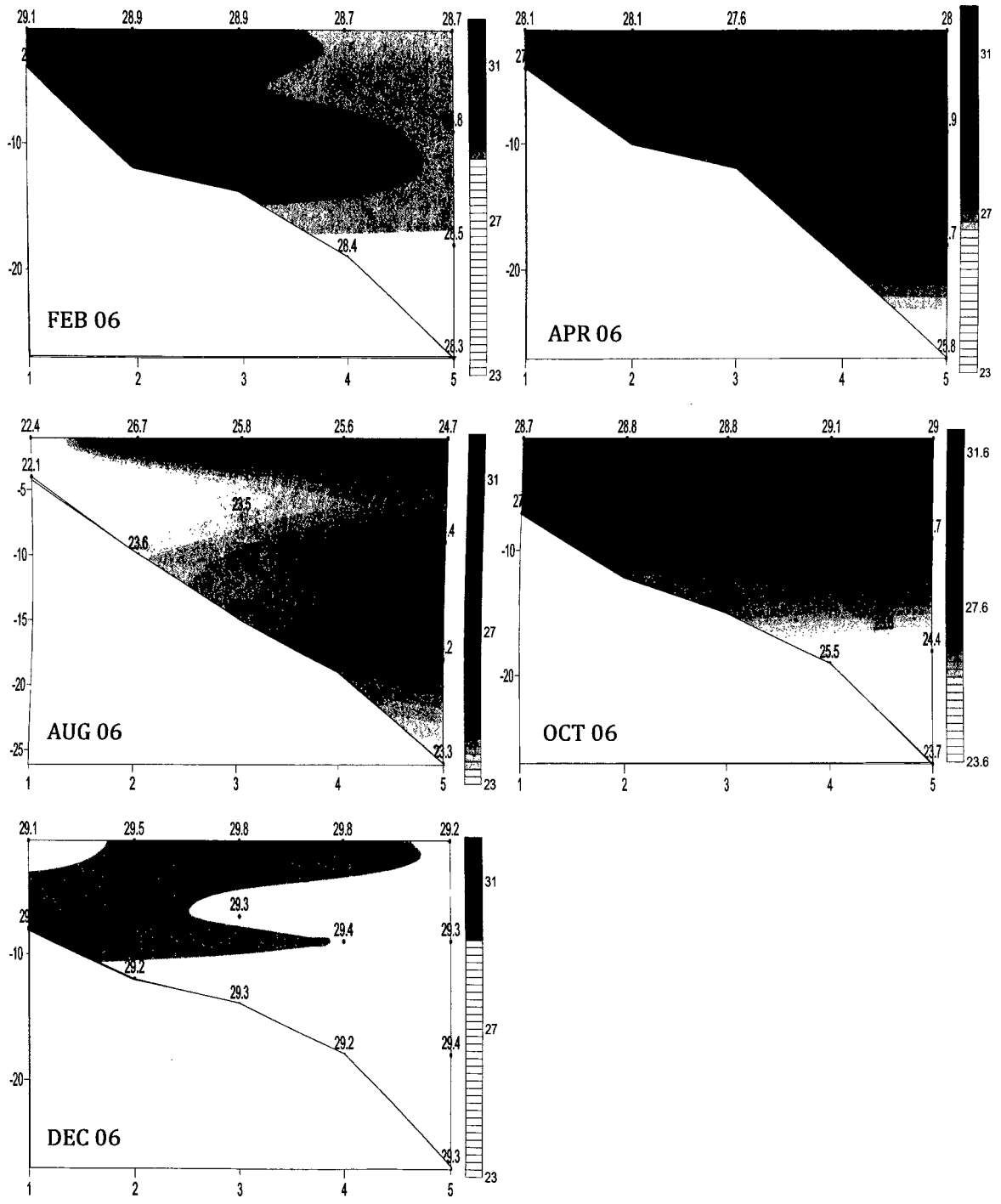


Fig. 4B.18c. Spatial and temporal variation of temperature (◦C) over the shelf off Goa during 2005-2006. X-axis denotes distance (Km) from the coast and Y-axis denotes depth (m).

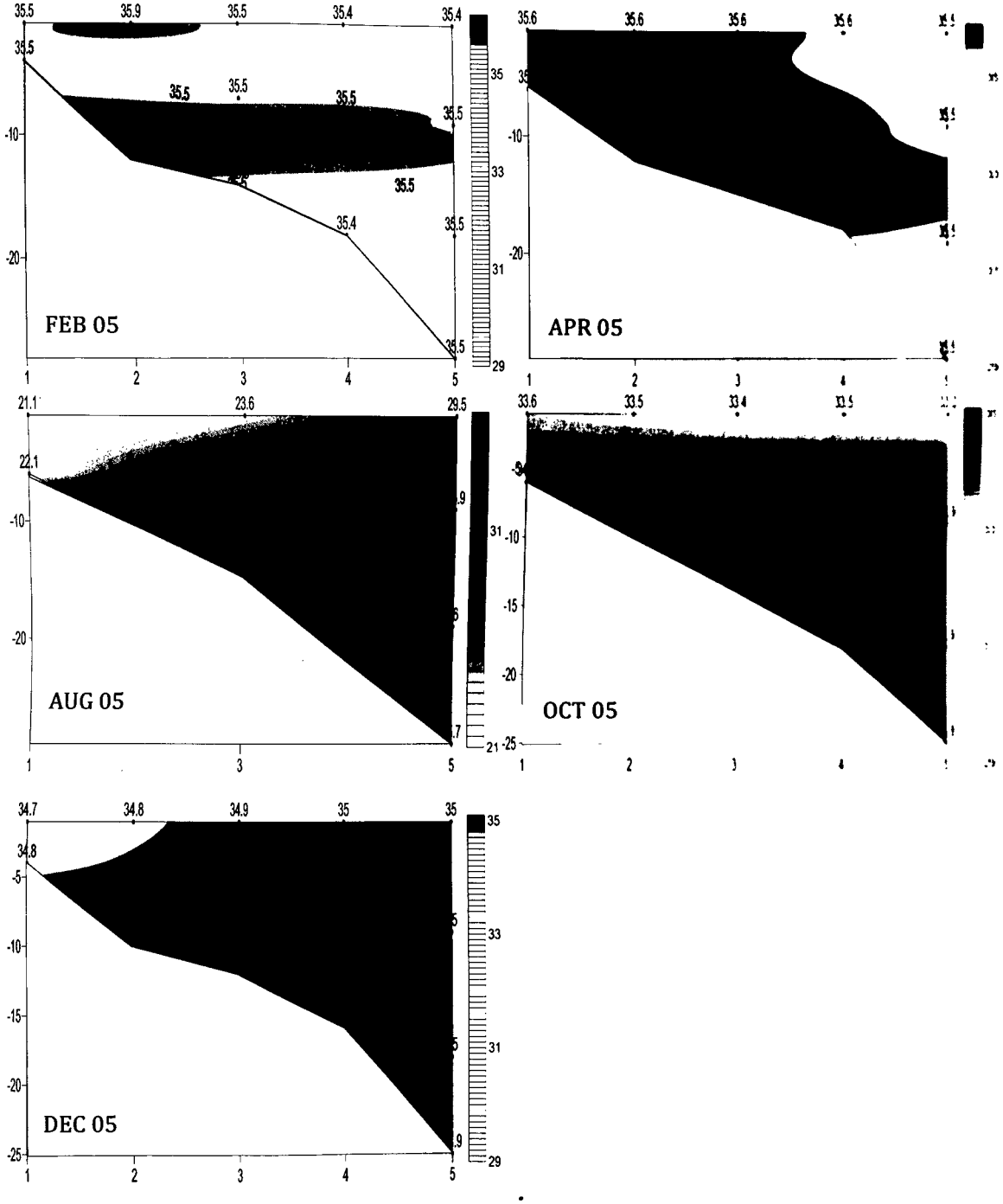


Fig. 4B.18d. Spatial and temporal variation of salinity (psu) over the shelf off Goa during 2005-2006. X-axis denotes distance (Km) from the coast and Y-axis denotes depth (m).

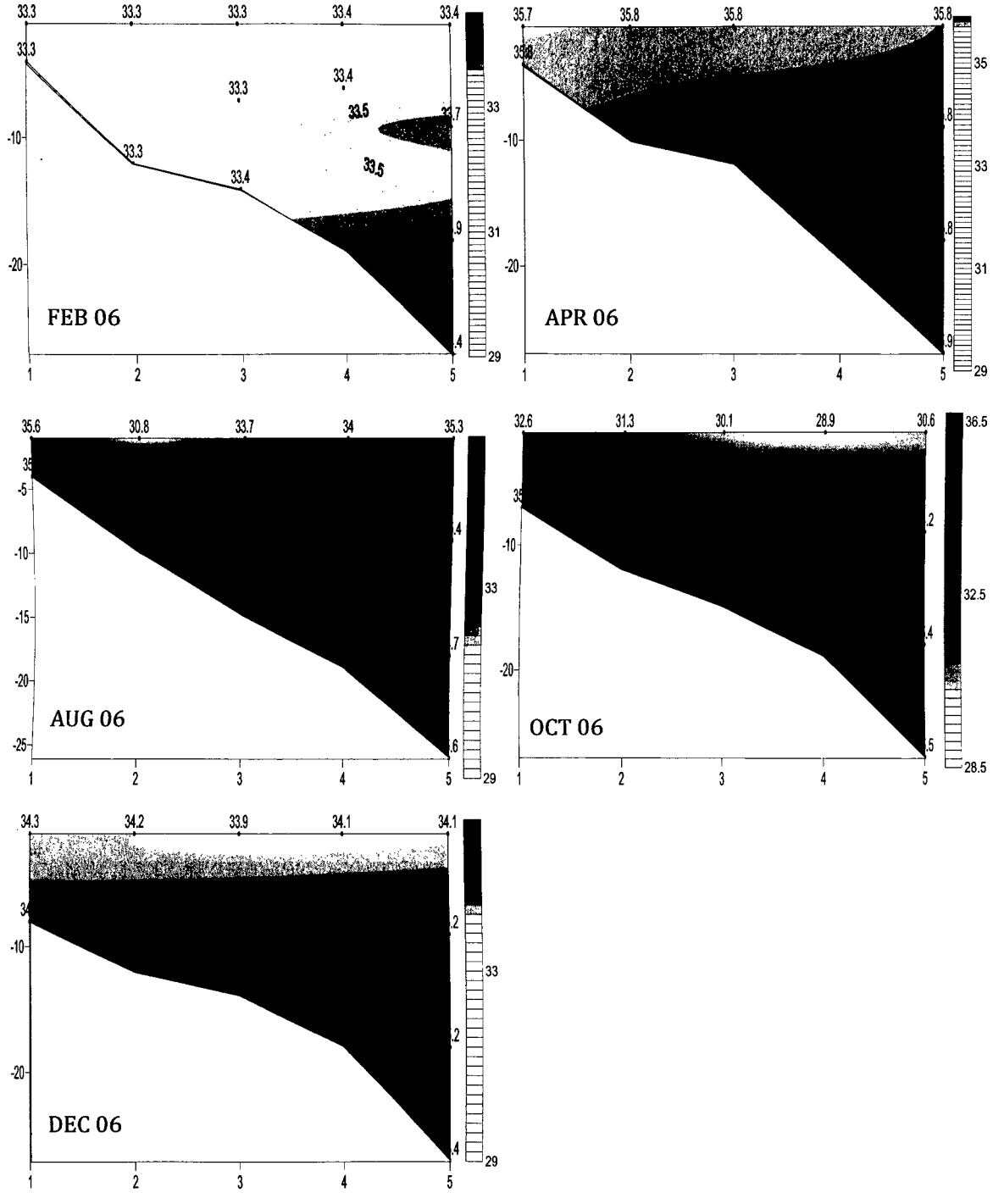


Fig. 4B.18d. Spatial and temporal variation of salinity (psu) over the shelf off Goa during 2005-2006. X-axis denotes distance (Km) from the coast and Y-axis denotes depth (m).

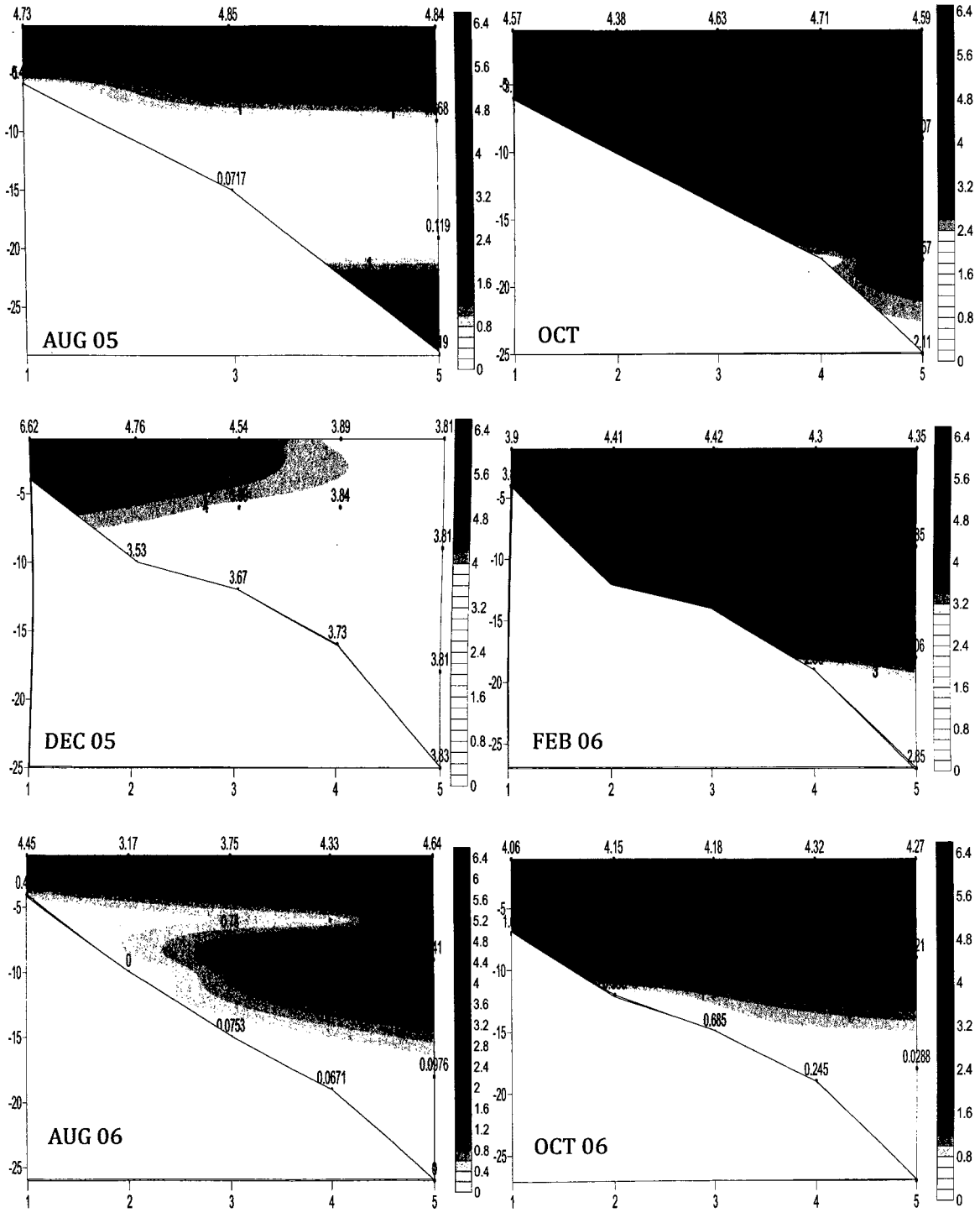


Fig. 4B.18e. Spatial and temporal variation of dissolved oxygen (ml L^{-1}) over the shelf off Goa during 2005-2006. X-axis denotes distance (Km) from the coast and Y-axis denotes depth

CHAPTER 5

MESOZOOPLANKTON COMMUNITY IN THE OXYGEN MINIMUM ZONE OF THE ARABIAN SEA

5.1: INTRODUCTION

The Arabian Sea (AS) experiences seasonally reversing monsoon winds. The winds blow from the northeast in winter (November-February; northeast monsoon) and from the southwest in summer (June-September; southwest monsoon). It is only during the intervening inter-monsoon periods, when the sea surface temperature (SST) increases to about 28°C, that the Arabian Sea acquires typically tropical character (Herbland and Voituriez, 1979). The southwest monsoon is the most important period for the region because the strong southwesterly winds force vigorous upwelling. The waters of upwelling along the coast of Somali, Yemen and Oman are carried over 1000km offshore resulting in the large scale nutrient enrichment of the central AS (Naqvi *et al.*, 2006) thus, making it one of the most productive region of the worlds ocean (Bauer et el., 1991; Kumar *et al.*,1996; Ryther *et al.*, 1996).

The northern Arabian Sea is one of the few regions in the open ocean where below thermocline water is severely depleted in dissolved oxygen with $0_2 < 0$ .1ml/l (Sewell and Fage 1948; Naqvi, 1987; Kamykowski and Zentara, 1990; Olson *et al.*, 1993; Morrison *et al.*, 1999). The Oxygen Minimum Zone (OMZ) is an oxygen-depleted layer of water, usually in 200 to 1000 m water depth. Although oxygen minimum zone waters constitute only about 0.1% of the total ocean volume in the world, 20-40 % of total oceanic nitrogen loss is estimated to occur therein. The distribution of open ocean OMZ is controlled by large scale ocean circulation. OMZ are permanent suboxic features of the oceanic water column that strongly influence zooplankton distribution and biogeochemical cycles.

Often OMZs support bacterial denitrification in which nitrate ions are used for oxidation of organic matter; in the process they are reduced to molecular nitrogen with nitrite as an intermediate (Codispoti and Christiansen, 1985; Naqvi, 1994). Richards (1965) showed that

several important biogeochemical changes are involved in the change from oxic to anoxic conditions. The first of these processes is that during oxygen depletion, facultative bacteria switch over to the use of nitrate ions for oxidation of organic matter. In the ocean, the major end-product is free nitrogen. This process, called denitrification, is a major component of the nitrogen cycle (Naqvi, 1994). The suboxic zones in the Arabian Sea comprise one of the three major water column denitrification sites in the world ocean (Codispoti, 1989) and have an annual denitrification rate of 10–30 Tg N yr^{-1} (Mantoura *et al.*, 1993; Naqvi *et al.*, 1992). After an almost complete removal of nitrate and nitrite from the ocean, sulfate ions serve as the next preferred reduction substrate, leading to production of hydrogen sulfide or true anoxic conditions. This stage is most often reached in bottom sediments and is rarely reached in the open ocean. Regions which experience denitrification but no hydrogen sulfide production are referred to as suboxic.These features make AS an interesting and almost unique area to analyze patterns of horizontal and vertical distributions of flora and fauna. The present investigation is based on the data collected onboard on RV *Roger Revelle* in 2007 during which most of the samples were collected. This expedition was undertaken during the southwest monsoon of the year 2007. The study area lies between Lat:14° S to 23° N ; Long: 56 to 73° E in the northern Arabian Sea and the cuise track is shown in the chapter 2 (Fig. 2.1). The cruise track begins from the inner shelf region on eastern coast of Arabian Sea, at the time-series location G5 (15 km off Goa coast) at 15° 31'N, 73 ° 39'E and sailed acrossed the northen Arabian Sea.

This study presents results obtained from few representative stations of the cruise transect located between latitudes 14.17° N to 15° N and longitudes 70° E to 58.39° E. The distribution of mesozooplankton, particularly calanoid copepods, under the influence of the OMZ is described in this chapter. The multiple plankton net (MPN) was deployed to obtain stratified samples from 5 depths of upper 1000mt (see below).

For convenience, sampling transect is divided into two regions based upon the hydrography of this region (i) eastern Arabian sea (east of 64°E; stns 2, 3, 4, 6, and 23 situated between latitudes 14.59 to 15° N and longitudes 70 to 63.59° E) and, (ii) western Arabian Sea (west of 64° E; stns 11, 12, 15, 16, 17 and 20 situated between Latitude:14.15° - 15.6 °N;

Longitude: 62° E to 58.39° E). The spatial variations in biomass and findings on horizontal and vertical pattern in assemblages are also reported. A brief investigation on phytoplankton composition and primary productivity of the mixed layer depth with regards to dissolved oxygen is not well understood and is briefly highlighted in this chapter.

5.2: MATERIALS AND METHOD

Samples were collected during a cruise aboard Roger Revelle (RR) during peak monsoon months, June-August, between 23 August and 16 September 2007 (Fig. 2.1). All water samples were obtained with a CTD – rosette sampler. Temperature-salinity (and fluorescence) data was obtained from Sea Bird CTD.

Routine chemical analyses of water samples for dissolved oxygen and nutrients were performed following standard procedures (SCOR, 1996) as mentioned in Chapter 3. Measurement of the biological parameters (Chlorophyll, phytoplankton and primary productivity) were done and processed following JGOFS Protocols (UNESCO, 1994; Grasshoff *et al.*, 1983; SCOR, 1996; see chapter 3). Zooplankton samples were obtained using a Multiple Plankton Closing Net (Hydro-Bios, mouth area 0.25 m^2, mesh size 2μm). Five stratified depths samples were collected from each station: 1000-500m, 500-300m, 300-base of thermocline (BT), BT-top of thermocline (TT) and TT-surface (mixed layer). Sample preservation followed by biomass estimation is detailed in chapter 3.

5.3: RESULTS

5.3.1: HYDROGRAPHY AND NUTRIENT CHARACTERISTICS

The monsoon cycle is a major factor, which influences the hydrographic features of the Arabian Sea. The period of our observation was during peak SWM (August-September, 2007). The region was characterized by moderate south-westerly wind (~7m s^{-1}), relatively low sea-surface temperature (~26 °C), and shallow mixed layer (~ 50-70m). During our cruise, strong

upwelling signatures were evident towards the Omani shelf, west coast of Arabian Sea, at stations (stns) 11, 12, 15, 16, 17 and 2 where the sea surface temperature in the upper 20mt depth was low and ranged from 24.9 to 26.6°C, least was recorded at stn 17 (Lat: 15° 47. 495 N and Long: 60° 15.01° E). On the other hand, the hydrographic characteristics at stn 2, 3, 6, 7 and 23 (Lat:15°N, Long: 70 to 63.59°E) were quite different from those stns located along the western Arabian Sea where sea surface temperature was found higher ranging from 26.9 - 27.8 5° C. However, there was little seasonal effect on the salinity ranging from 35.7 to 36.6 psu at stn 17 and stn 3, respectively. However, there was large difference in dissolved oxygen concentration both horozontal and vertical scale in the AS. Generally, dissolved oxygen concentrations was below 0.2 ml L^{-1} between 200 and 1000 m. Even lower concentrations (close or below the detection limit between 150and 500m) were recorded at some stations (e.g. Sta. 23). However, at few locations hypoxic conditions prevailed (O_2 = 21 μM or 0.47 ml L^{-1}) within the euphotic zone (e.g. stn 11, 12). On the whole, the oxygen concentrations in the top 100 m varied from 162 μM to 20 μM along the eastern transect (stn 6 and stn 3, respectively). Whereas, concentrations were higher (155-9 μM) in the upwelling affected waters of stn 12 and 17. These findings suggest that sampling transect covered regions of open ocean that were also under the strong influence of upwelling.

Similarly, nutrient levels towards the east coast of Arabian Sea were low compared to western part. A number of stations occupied during the cruise (east of 64°E) were comparatively oligotrophic (i.e. surface nitrate was below the detection limit (< 0.2 μM). For instance, at stn 2, nitrate at near surface (upper 10m), was almost negligible, however apart from this station nitrate values ranged between 0.3 and 4.8 μM. These low and high values were detected at stn 4 and 23, respectively. While, the nitrate values towards the mid-west coast varied from 1.6 to 11 μM. The highest nitrate concentration was recorded in surface was at stn 12. Such high surface values are peculiar during the SW monsoon in the western Arabian Sea. Vertically, nutrient concentration increased with depth (data not shown here) reaching a maximum level of around 40 μM in the twilight zone. At times higher concentrations were recorded well within the euphotic zone.

5.3.2: CHLOROPHYLL *A*

Phytoplankton biomass (chl *a*) in the euphotic zone during the sampling period varied between 0.01 and 0.78 mg m^{-3} (avg. 0.22± 0.21 mg m^{-3}). Waters of the western region were with higher chl *a* biomass that varied between 0.01 and 0.78 mg m^{-3} (avg. 0.26± 0.24 mg m^{-3}). This high value was detected in shallow waters of stn 12 (ca.100m). Conversely, highest concentration in the eastern transect was at stn 6 (20m) depth with a concentration of 0.5 mg m^{-3}. Integrated chl biomass (0-100 m) ranged from 9.27 to 27.8 mg m^{-2} (avg. 20±6.7mg m^{-2}) with a maximum at stn 17 and the least was at stn 4. Further, higher biomass was also recorded at stns 20 and 12 with 27 and 26 mg m^{-2} respectively as compared to eastern region of the Arabian Sea.

5.3.3: PRIMARY PRODUCTION

Primary productivity (PP) measurements in the euphotic zone were also carried out at selective stations by following in-situ ^{14}C-technique, which was carried out at stns 4, 8, 11 and 16. The PP (mg C m^{-2} d^{-1}) ranged between 720 (stn 4, eastern AS) and 2565.6 (stn 16; western AS). The productivity measurements below 80m depth showed a substantial decrease roughly coinciding with low dissolved oxygen possibly low DO inhibits autotrophic productivity rates (Fig. 5.1).

A comparative data on phytoplankton (primary production and biomass) and dissolved oxygen was compiled to get a better insight on the effect of dissolved oxygen on phytoplankton prevailing in the Arabian Sea. Interestingly, the primary productivity (PP) in the upper 4 m depth at most of the stations was ca. 95% in oxic waters (>3ml L^{-1}). This zone was found to support chlorophyll concentration between 49 and 88% of the total standing stock. At mid depths (60-80 m), the productivity decreased to less than 5%. Interestingly, phytoplankton biomass at this depth was sizable with 49, 10, 16, and 35% at stn 4,8,11 and 16, respectively. Below this depth (100-120 m) production and standing stock of chlorophyll reduced drastically (<5%), possibly under the influence of low light and hypoxia.

5.3.4: PHYTOPLANKTON COMPOSITION IN THE EUPHOTIC ZONE ABOVE OMZ

Overall, a total of 74 species belonging to 41 genera were recorded from the euphotic zone of the central Arabian Sea. Higher diverswity was recoreded along the western transect (Table 5.1). Phytoplankton density and composition along these two transect (eastern and western) is detailed below. The vertical distribution of the total phytoplankton abundance is shown in Fig. 5.1.

EASTERN TRANSECT: The phytoplankton abundances were low within the euphotic zone of stns. 2, 3, 4, 6 and 23 and was characterized by low chlorophyll a values (0.001 to 0.52) suggesting oligotrothic conditions. This high chlorophyll value was recorded at shallow depth of stn 6. In general, phytoplankton were found to decrease with depth. Numerically, phytoplankton abundances varied between 144 and 86004300 cells L^{-1}. This exceptionally high cell abundance in the surface waters at stn 23 was due to the presence of *Pheocystis globosa* bloom (15°N and 63.59°E). The presence of Phaeocystis colony (99%) was an exceptional case. Generally, diatoms were predominant forms of phytoplankton (>5 μm) accounting >95% followed by dinoflagellates. Most of the phytoplankton were found in near surface water, and negligible below 60m water depth. A total of 53 species belonging to 32 genera of phytoplankton community were identified from these stations. Of which, 32, 18, 2 and 1 species were respectively belong to diatoms, dinoflagellates, silicoflagellates and prasinophytes. The pennate diatoms were roughly two times higher than the centric forms (Table 5.1). In total, contribution of silicoflagellates was negligible (<2%) that were found restricted to lower part of the euphotic zone (ca.40-60m). Dictyocha spp. belonging to silicoflagellate was found only at sub-surface depths. The most common diatoms along this transect were *Rhizosolenia spp., Cosinodiscus* spp., *Navicula* spp., *Pseudonitzchia* spp., *Nitzschia* spp., and *Thalassiosira* spp. Dinoflagellate community was represented by *Pyrophagus spp, Ceratium spp, Dinophysis spp., Gymnodinium* spp., *Gyrodinum* spp., *Prorocentrum* and *Protoperidium* spp. The list of phytoplankton assemblage is shown in the Table 5.1. An endocytic association of *Leptocylindrus mediterraneus* with *Solenicola setigera* was also recorded at stn , see Chapter 6.

Remarkably, amongst the eastern transect stations; the most diverse phytoplankton was at stn 23 with a total of 30 species. Diatom composition contributed >85% to the total phytoplankton and was represented by species belonging to the genus *Rhizosolenia, viz. R.alata, R. imbricata.R. hebetata, R. robusta, R. setigera, R. shrubsolei, R. stolterfothii* and *R.styliformis.* Interestingly, *Amphisolenia bidentata* (dinoflagellate), an upwelling indicator species was also found at shallow depths (ca. 20m); (Plate I-VII)

WESTERN TRANSECT: The phytoplankton abundance and composition was slightly high towards the western transect of Arabian Sea (stns 11, 12, 15, 17, 16 and stn 20) in comparison to eastern region. Chlorophyll *a* was low and ranged between 0.001 and 0.78 mg m^{-3}. This high chlorophyll value was recorded at shallow depth of stn 12. Lower values of standing stock were observed at lower half of the euphotic zone (ca.100m) at most stations. At this region cell abundance of phytoplankton varied from 56 to 21602592 this high abundance was again due to the thick bloom of *Pheocystis* occured at stn 20 (15.6 °N; 6 °E) (Fig. 5.2). The lowest cell counts were observed at stn 17.

A total 73 species belonging to 33 genera of phytoplankton were identified from stations of this region. Of which, 47, 23, 1 and 1 species were belong to diatoms, dinoflagellates, silicoflagellates and Prasinophyte, respectively. Diatom composition to the total autotrophic community was larger (>90%) than that of the eastern region. Overall, stn 16 and 17 had the most diverse forms with 9 species belonging to the genus *Rhizosolenia, viz R. alata, R. imbricata. Rhizosolenia curvata, R. hebetata, R. robusta, R. setigera, R. shrubsolei, R. stolterfothii* and *R. styliformis.* This was followed by the genus *Chaetoceros viz* (*C. peruvianus, C . messanensis , C. coarctatus, C. loranzianus, C. decipens*). Similarly, amongst dinoflagellates, the most common forms present were of the genus *Ceratium* (*C. fusus, C. furca, C. kofoidii, C. lineatum, C. macroceros, C. horridum* and *C. tripos*), Prorocentrum (*P. Protoperidinium* (*P. depressum, P . granii, P. oceanicum, P. stenii)* .The list of phytoplankton community is shown in Table 5.1 *Dictyocha fibula* was the only species belonging to Silicoflagellate group of the total phytoplankton community. Dinoflagellates *Amphisolenia bidentata,* and a few dinoflagellate cysts were also commonly observed at these locations. In addition, unusual epiphytic associations of *Cheatoceros coarctatus* with *Vorticella oceanic* and *Rhizosolenia alata* with

zoothamnium colony were also reported in this region particularly at stn.17 (15.47°N and 6 .15 °E). Vertical and spatial distribution of various phytoplankton taxa are presented in Fig. 5.3.

Overall, the phytoplankton data suggests that the western region is more productive during this (SWM) season and is under the strong influence of upwelling during south west monsoon.

5.3.5: HORIZONTAL AND VERTICAL DISTRIBUTION OF ZOOPLANKTON ACROSS AN OXYGEN GRADIENT OF THE ARABIAN SEA

One of the key objectives of this study was to characterize zonation of mesozooplankton species relative to oxygen gradients. To address this question, only day time samples collected during the cruise were considered to study resident species of deep water forms permanently/temporarily inhabiting the OMZ. Station data processed for this study include stns 3, 6 and 23 represented the eastern region and stns 12, 15, 17 and 2 represented western part.

The zooplankton biomass, abundance and composition were studied from upper 1000m depth. Along the east-west transect strong gradients in OMZ was recorded. Numerically their abundance did not differ much between eastern and western region. Nevertheless, along the east coast, high and low biomass was recorded respectively at stn 23 and at stn 3. Alternatively, higher biomass was recorded at stn 12 (and low values at stn 15) in the western transect. The zooplankton composition data is compiled along the oxygen gradient in the water column and is given below. These gradients are classified based on the levels of dissolved oxygen concentration in the water column and is designated as (a) oxic (>2ml L^{-1}), (b) hypoxic (<2- 0.2 ml L^{-1}) and (c) suboxic (< 0.2ml L^{-1}) zone.

5.3.5.1: OXIC ZONE: In the upper mixed layer depth (MLD) or above the thermocline (TT) less than 100m depth where dissolved oxygen is normally >2ml/L (160µM to 80µM).The overall abundance in these zones ranged from 236 to 4224 org /m^3. This high abundance was recorded in the eastern side of the study area, particularly at stn 23 (biomass: 240ml/100m^3, 0- 20m). Lowest

abundance (236 org/m^3 (biomass: 5.3 ml/100m^3) was found at stn 3 (0-60 m; 15°N and 67.53°E). In the western side zooplankton density varied between 1797 and 3492 org /100m^3.

Similarly, high biomass (0-35m; 125 ml/100m^3) and abundance (3492 org/m^3) was recorded at stn 12 (16.12 °N and 59.41°E) and least (72 ml/100m^3) was at stn. 15 (0-50m;14.15°N and 58.39°E).

Among zooplankton, copepods generally dominated numerically at all stations but, at stn 200 (15.6° N and 62° E)) spectacular swarms of *Pyrosoma* replaced the dominance of copepods. These swarms are filter feeders, free floating and confined at the sea surface. The copepods present in the oxic strata (>2ml L^{-1}; MLD and top of thermocline layers i.e. upper 70 -80mts) were *Acartia* spp., *Paracalanus* spp., *Acrocalanus* spp., *Clausocalnus* spp., *Cosmocalnus* sp., *Canthocalanus* sp., *Calocalanus* sp., *Undinula* sp., *Eucalanus* spp, *Calanopis* spp, *Candacia* spp., *Labidocera* spp., *Lucicutia* sp.,*Centropages* spp, *Temora* spp, *Scolecithricella* spp., *Eucheata* spp., *Calanoides* sp., *Corycaceus* spp., *Farranula* spp, *Oncea* spp, *Copilia* spp., *Oithona* spp, *Clytemnestral* spp. Other non- copepod groups present were Amphipods, Ostracods, Decapods, Cheatognaths, Larvacea, Doiolum spp, Gastropoda and polycheates. The list of mesozooplankton community is shown in Table 5.2 Of these copepods, *Paracalanus* spp, *Acrocalanus* spp, *Cosmocalanus darwinii., Eucalanus subcrassus, Oithona plumifera, Oncea* spp, and *Corycaceus* spp contributed >2% in abundance in this oxic strata. Few representative taxa present in oxic zone of the water column are shown in the Fig. 5.4

5.3.5.2: HYPOXIC ZONE: The oxygen level between bottom of the thermocline (ca. 60m) to a depth of 300m generally varied between 88 and 10μM (<2- 0.2 ml L^{-1}). Mesozooplankton abundance in this zone along the eastern transect varied from 15 to 43008 org/m^3 (and biomass between 2.3 and 60 ml/100m^3). The high abundance and biomass was recorded at stn 3 (43008 org/m^3 and 60ml/100m^3; strata: 60-120m) while lower values were recorded at stn 6 (strata: 8 -25 m) with abundance of 15 org/m^3 and biomass of 2.3 ml/100m^3. Conversely, in the western side high abundance and biomass was recorded was at stn. 17 (50-100m; 329 org/m^3 and 32 ml/100m^3) and least at stn 15 (strata: 50-120m; 130 org/m^3 and 3.4 ml/100 m^3).

The mesozooplankton community existing in this zone were *Calanus* spp, *Cosmocalanus* sp, *Calanopis* sp, *Eucheata* spp, *Eucalanus* spp, *Rhincalanus* spp., *Lucicutia* spp., *Calanoides carinatus, Pleuromama* spp., *Scolecithrcell* spp, *Heterorabdus* spp, *Aetidues* spp, *Euchirella* spp., *Mormonilla phasma, Corycaceus* spp, *Oncea* spp, *Oithona* spp. Other non-copepods forms present were belong to Ostracoda, Decapoda (*Euphausid* spp), Chaetognatha (*Sagita* spp), Doliolum spp. Gastropoda, and Polycheata. The list of mesozooplankton community present in hypoxic waters is shown in Table 5.3.

Amongst these forms, the dominant copepods contributing >2% to the total abundance were *Pleuromama indica, Pleuromama graciles, Rhincalanus nasutus, Rhincalanus cornutus Eucalanus attenatus, Lucicutia flavicornis, Lucicutia ovalis, Oncea* spp, *Oithona* spp., *Gastropods* and *foraminiferans* were also commonly recorded in the hypoxic waters.

5.3.5.3: SUBOXIC ZONE: At deeper depth (ca 300-1000m) zooplankton biomass (and abundance) was relatively lower. The dissolved oxygen level at this depth was below 4.5μM suggesting influence of low oxygen on this organism. Decreasing biomass with low oxygen waters was seen in both western and eastern transect (see Fig. 5.5). The mesozooplankton abundance in the eastern region coast varied between 3.9 to 1564 org/m^3 and biomass between 0.2 and 6.7 ml/100 m^3. This high abundance and biomass was recorded at stn. 23 (170-1000m). While, least was recorded at stn 3 (120-1000m). In the western transect, high abundance (162 org/m^3) and biomass (5.5 ml/100m^3) was recorded was at stn 12 (200-500m). Lowest population density (8 org/m^3) of this community was at stn15 (120 -1000m) with a biomass value of 0.53 ml/100m^3.

Distribution of copepods vertically showed distinct differences possibly driven by the levels of dissolved oxygen concentration. However, the deep water copepod species of zooplankton community commonly inhabiting suboxic waters at deeper strata consisted of *viz. Eucalanus attenatus, Eucalanus, Rhincalanus cornuta, Rhincalanus nasutus, Lucicutia maxima, Lucicutia clausii, Pleuromama indica, Heterorabdus abbysalis, Mormonilla phasma, Mormonilla minor, Oncea* spp, *Oithona* spp and *Clytemnestra scutellata.* Ostracods, *Euphausiid, Sagitta* sp, *Salp,* and myctophids dominated among non copepod forms. Interestingly, a mid

water shrimp, *Ophophorus typus* and mysid, *Gnathophausia* sp. were also found to occur in sub oxic waters. The list of mesozooplankton community recorded in suboxic waters is shown in Table 5.4.

It may be noted that, often there is an overlap of mesozooplankton community distribution within hypoxic and suboxic zone as most of these species exhibit diel migration as reported in the previous studies. Few representative taxa prevailing in core of OMZ region are shown in Fig. 5.6

5.4: DISCUSSION

Indian Ocean contains one of the most pronounced OMZ which most intense in the northern sector (Naqvi, 2006) of which two-third of the global continental margin area is affected by natural oxygen deficiency (<0.2ml L^{-1}) in the water column. The dissolved oxygen concentration in the surface layer is high (>3 ml L^{-1}) but decreases sharply across the pycnocline. At the intermediate depths (200-1000m) the oxygen levels remain low before it increases towards the bottom again. The oxygen deficiency makes a profound impact on the biological processes (Morrison *et al.*, 1999). Primary production (PP) is largely controlled by the availability of nutrients, particularly, nitrate (Naqvi *et al.*, 2003) and can further be limited by iron (Fe) deficiency (Naqvi *et al.*, 2010). The quantity and quality of primary production are affected when suboxic waters ascend to the euphotic zone (Naqvi *et al.*, 2006). During this season, surface waters were enriched with high nutrient concentration (NO_3^{3-}) ca. 11μM. The recent findings of the study have shown that primary productivity in open waters sustained high production with >2.6 g C m^{-2} d^{-1} in the euphotic zone. Earlier studies have also shown high productivity in the western Arabian Sea (Smith and Codispoti, 1980). But, such production is restricted to the thin oxygenated layer, even though it has sufficient nitrogenous nutrients due to the unsuitability of the oxygen depleted environment for the growth of oxygenic photosynthesizers (Naqvi *et al.*, 2010). But what is novel is that >95% primary production occur in the oxic waters (>3ml L^{-1}), it is further dwindles to ca. 5% in mid depths and in hypoxic/ suboxic waters >100m, the productivity drops to ca. >1 % (PP plots). Similar PP profiles were

also reported from the coastal waters of eastern Arabian Sea (Naqvi *et al.*, 2010). Likewise, most of the phytoplankton biomass (Chl *a*) (>95%) subsist in upper 60m depth. Several studies have also been carried out on temporal and spatial variation of primary productivity and phytoplankton biomass in the Arabian Sea (Bhattathiri *et al.*, 1996; Gauns *et al.*, 2005). Study on changes on biological and physico-chemical parameters were examined during various stages of upwelling during SWM in the south-eastern Arabian Sea (Haeebrehman *et al.*, 2008) found that phytoplankton were dominated by *Nitzschia seriata* and *Rhizosolenia alata* (pennate diatoms). Smith and Codispoti (1980) reported *Nitzschia delicatissma* and *Rhizosolenia styliformis* were characteristics species in the upwelling region off Somalia. In the same way, several species of taxa *Rhizosolenia, Ceratium, Nitzschia, Prorocentrum, Protoperidinium* were predominant forms prevailing in the Northern Arabian Sea (Table 5.1). Apart from these species, bloom of *Phaeocystis globosa* was found occasionally. Dinoflagellates, *Amphisolenia bidentata,* a characteristic indicator species of upwelling waters were also significant. In addition, epiphytic associations of *Cheatoceros coarctatus* with *Vorticella* sp and *Rhizosolenia alata* with *zoothamnium* colony and endocytic associations of *Leptocylindrus mediterraneus* with *Solenicola setigera* were also reported from the study region (see Chapter 6).

OMZ are very difficult places to survive, with extreme harsh conditions and ill-suited for supporting life affecting its physiology and life cycle, and also the pattern of distribution and migration (Diaz and Rosenberg, 1995; Childress and Thuesen, 1993; Ekau *et al.*, 2009). And because there is no light for photosynthesis far beneath the surface, animals have to depend on the few food particles that sink from the productive upper ocean. We speculate that the occurrence of colonial swarm of *Pyrosoma spinirostrum* in the near surface waters at stn 20 is probably one such example wherein the OMZ below restricted its vertical migration.

However, some organisms have modified metabolic systems for surviving in the OMZ (Childress and Seibel, 1998). The survival of oceanic organisms particularly zooplankton in this zone depends on their oxygen demand and the capacities for oxygen extraction and transport, anaerobic ATP production and metabolic suppression are required for daytime into most extreme OMZ (Escribano, 2006, Gonzalez and Quiñones, 2002). The ability of certain zooplankton species to transit through low oxygen environments is also due to the presence and activity of

enzymes *i.e.* lipid dehydrogenase (LDH) (Escribano, 2006). For example, in *Euphausid* sp. living in the OMZ (Gonzalez and Quiñones, 2002). Apart from crustaceans, recent observations indicate that some species of hydromedusae (Rutherford and Thusen, 2005) and ctenophores (Thusen *et al.*, 2005) can also tolerate low oxygen conditions. The presence of a few species belonging to families Metridinidae, Auguptiliidae, Eucalanidae, Heterohabidae, Lucicutiidae and Mormonillidae, Onceadae were the characteristics species of this poorly oxygenated (<4.5 μM) mid depth core of the OMZ. Apart from these copepode species, mid water shrimp *Ophophorus typus* and mysid, *Gnathophausia ingens* were also found to occur. The latter species has unusually wide respiratory range, which enable them to persist solely within deep low oxygen layers (Mincks *et al.*, 2000). Copepods like *Pleuromamma indica* can migrate in and out of the OMZ of the Arabian Sea where oxygen can be as low as 0.1ml/l (Saraswathy and Iyer, 1986; Smith, 1982). *Lucicutia grandis* is a good indicator for the lower OMZ interface of the Arabian Sea and Eastern Tropical Pacific (Gowing and Wishner, 1992, 1998; Wishner *et al.*, 1995; Saltzman and Wishner, 1997 b; Morrison *et al.*, 1999).

The seasonal ontogenetic migrants, *Calanoides carinatus* and *Eucalanus subtenuis* (Smith *et al.*, 1998, 2001) were distinct species during summer upwelling, found abundantly within the euphotic zone. The former species was found restricted only to the western region and were not found along the eastern Arabian Sea (Fig. 5.6) for the reason unknown.

5.5: CONCLUSIONS

In the Arabian Sea mesozooplankton distributions showed strong relationships to the oxygen profiles. The mesozooplankton community responses to the OMZ varied with the type of organism. Primary production decreased substantially in low oxygen waters. Overall, the western Arabian Sea showed higher biomass and abundance compared to the eastern region owing to the peculiar hydrography and strong winds during SWM consequently inducing strong upwelling signatures of which were reflected in the study region.

Table: 5.1 Spatial composition of phytoplankton in the eastern and western Arabian Sea

Eastern transect	**Western transect**
DIATOM (Centric)	**DIATOM (Centric)**
Melosira spp	*Actinopticus undulata*
Cheatoceros spp	*Bacteriastrum delicatulum*
Coscinodiscus spp.	*Chaetoceros messanensis*
Eucampia zodiacus	*Chaetoceros pervuainus*
Guinardia flaccida	*Chaetoceros* spp.
Guinardia striata	*Cheatoceros coarctatus association* with *vorticella*
Leptocylindrus mediterraneus association with Solenicola setigera	*Cheatoceros loranzianus*
Melosira sulcata	*Corethron hystrix*
Planktonella sol	*Coscinodiscus radiatus*
Thalassiosira spp	*Coscinodiscus* spp.
Rhizosolenia spp	*Cosinodiscus eccentricus*
Rhizosolenia alata	*Cosinodiscus marginatus*
Rhizosolenia hebetata	*Cyclotella striata*
Rhizosolenia imbricata	*Guinardia flaccida*
Rhizosolenia robusta	*Guinardia striata*
Rhizosolenia shrubsolei	*Leptpcylindrus minimus*
Rhizosolenia spp.	*Planktonell sol*
Rhizosolenia styliformis	*Streptotheca tamensis*
	Thalassiosira subtilus
	Thalassiosira spp
	Rhizosolenia alata
	Rhizosolenia association with *Zoothamnium* sp.
	Rhizosolenia curvata
	Rhizosolenia hebatata
	Rhizosolenia imbricata
	Rhizosolenia robusta
	Rhizosolenia setigera
	Rhizosolenia shrubsolei
	Rhizosolenia sloterforthi
	Rhizosolenia spp.
	Rhizosolenia styliformis

DIATOM (Pennate)	***DIATOM (Pennate)***
Coconeis spp	*Asteromphalus* spp
Fragillaria spp	*Cocconeis* spp
Navicula delicatula.	*Fragilaria* spp
Navicula septentrionalis	*Fragillaria oceanica*
Navicula directum	*Navicula directum*
Navicula spp.	*Navicula* spp.
Nitzschia closterium	*Navicula transitrans f.delicatula*
Nitzschia spp	*Nitszchia closterium*
Pleurosigma spp	*Nitzschia* spp
Pleurosigma elongata	*Pleurosigma directum*
Pseudonitszchia spp	*Pleurosigma* spp.
Pseudonitzschia seriata	*Pseudonitszchia* spp
Thalasiothix spp	*Thalasiothix* spp
Thalassiothrix longissima	*Thalassionema nitzchoides*
	Thalassiothrix longisima
	Thalassiothrix spp
DINOFLAGELLATES	***DINOFLAGELLATES***
Scripsiella spp	*Amphisolenia bidentata*
Alexandruim spp	*Amphidinium* spp
Amphidinium spp	*Ceratium furca*
Amphisolenia bidentata	*Ceratium fusus*
Ceratium azorium	*Ceratium horridum*
Ceratium fusus	*Ceratium kofoidii*
Ceratium tripos	*Ceratium lineatum*
Dynophysis caudata	*Ceratium macroceros*
Gonulux	*Ceratium tripos*
Gymnodium spp.	*dinoflagellate cyst*
Gyrodinium spp	*Goniodoma* spp
Ornithoceros spp	*Gymnodium* spp.
Podolampus spp	*Gyrodinium* spp
Prorocentrum danae	*Phalacroma rotundatum*
Protoperidinium depressum	*Prorocentrum danae*
Protoperidinium spp.	*Prorocentrum micans*
Protoperidinium steinii	*Prorocentrum minimum*
Pyrophagus holorigicum	*Prorocentrum* spp
	Protoperidinium depressum
	Protoperidinium grani

	Protoperidinium oceanicum *Protoperidinium* spp. *Protoperidinium steinii* *Pyrophagus holorigicum*
CHRYSOPHYCEAE Phaeocystis globosa **SILICOFLAGELLATE** *Dictyocha* spp	**CHRYSOPHYCEAE** *Phaeocystis globosa* **SILICOFLAGELLATE** *Dictyocha* spp

Table: 5.2: Spatial composition of mesozooplankton in the oxic waters of the eastern and western Arabian Sea.

Oxic Zone	**East transect**			**West transect**			
Station	Stn 3	Stn 6	Stn 23	Stn 12	Stn15	Stn 17	Stn20
Biomass ml/100 m-3	5	125	144	126	72	96	73
Hydrozoa							
Siphonophorae	107						
Diphyes spp.			1280				
Medusae	640						
Trachy medusae	107						
Globeginia							3413
Gastopoda		6400	2702		1024	512	3413
Pelecypoda		1280	142				1707
Polychaeta	213	1280	1422	1461	1024	512	853
Copepode nauplii	107	1280	4124			3072	4267
Juvenile copepod	533		11804		4096	2560	
CALANOIDA							
Acartia spp	107	2560	284	2922	5120	3072	
Acartia erythrea			1564	1461	46	1024	1707
Acartia amboinenensis			2560				
Paracalanus sp.	960	6400	30293	46758	102400	54784	52053
Acrocalanus sp			13084	11689	13312	256	853
Acrocalanus gibber			6400		1024	1024	853
Calanus sp	533				2048		
Metacalanus sp					2048		
Clausocalanus spp	1600	39680	1422	4384	8192	2048	274
Cosmocalanus darwini		1280	1138	11689	2048		6827
Canthocalanus pauper	107		142		1024	128	1707
Calocalanus pavo			2844	1461	1024		1707
Undinula vulgaris	640	5120	1422		3072	1024	
Eucalanus attenatus	107	1280	284	1461	1024	1536	853
Eucalanus subcrassus.			142			14336	282
Eucalanus mucronatus	107						
Eucalanus elongatus					2048		
Eucalanus spp	533	11520	5404	51142	2048	2560	4267
Rhincalanus cornuta			2702				
Rhincalanus nasutus						512	
Neocalanus gracilis			1280				
Lucicutia flavicornis	747		1849	2922		2048	

Lucicutia ovalis			142				
Pleuromamma spp	533			1461		512	
Scolecithricella emarginata	427						
Scolecithricella spp			1564		1024		
Scolecithrix ctenopus			142				26
Scottocalanus helenae						512	
Scotocalanus spp		1280					
Scaphocalanus spp					1024		
Halioptilus				5845			
Heterorabdus sp							853
Aetideus sp					2048		
Macrosetella gracilis			142				
Euchirella maxima	107						
Centropage furcatus		2560	2560	1461	1024	2048	853
Centropages sp					1024		
Temora turbinata				1461			26
Temora discaudata					2048	512	
Temora stylifera			284	2922		512	
Calanopia minor		2560		5845			
Calanopia sp			1564	1461	2048		853
Candacia pachydactylus	107	1280				512	
Candacia curta			1280				
Candacia		2560	284		44		853
Labidocera acuta			1280				
Labidocera minuta			107				
Labidocera sp.			3840	1461		512	
Eucheata concinna	213						
Eucheata marina	1707	2560	142	1461			
Eucheata wolfendii	213		71			512	
Eucheata plana	107						
Euchaeta spp		8960	142		1024		
Undeuchaeta spp			71				
POICILOSTOMATATOIDA							
Corycaeus catus			1422	1461	1024	512	
Coryceus specious		1280					
Corycaeus sp.	960	20480	30151	33607	15360	14848	10240
Faranula gracilis			1280				
Faranula sp		7680	6400	1461			853
Vettoria granulosa			142				

Oncea venusta	427				46		
Oncea specious	427	83200					
Oncaea sp.	3947		39111	64292	44032	31744	52907
Copilia vitrare	107						
Copilia mirabilis		1280	5120	1461	1024		
Copilia sp		3840					
Sappharina gastrica						512	
Sappharina metallina			284				
Sappharina darwinii			142				
CYCLOPIODA							
Oithona plumifera	427		1991	37991	4096	1536	4267
Oithona spinirostris							137
Oithona sp.	1920	7718	8391	8767	5120	6144	5120
HARPACTICOIDA							
Clytemnestra scutellata		2560	142	1461		512	853
Euterpina acutifrons			1422			512	
Microsetella rosea				1461			853
Macrosetella gracilis			142				
Microsetella norvegica						512	
Amphipoda	213	2560		1461	2048	512	853
Ostracoda	853	1280	1564	4384	1024	2560	7680
Decapoda	427	3840	5404	1461	1024	2560	
Decapoda larvae							
Euphausiid spp.	213						2560
Lucifer typus		2560	1280				
Lucifer sp		1280					
Chaetognatha							
Sagitta sp	1173	35840	11804	18995	7168	13312	11093
Urochordata							
Oikiopleura sp.	1280	17920	8249	7306	2048	2560	1707
Salpa sp.		1280	3840				
Pyrostremma spinosum							51
Thalia			427				
Doliolum sp.	213		1707	2922	1024	4096	
Chordata							
Fish eggs		1280	1849			512	
Fish larvae	107		1280				853
Total org/100m3	**23573**	**298278**	**241458**	**349224**	**248968**	**180096**	**191942**

Table: 5.3: Spatial composition of mesozooplankton in the hypoxic waters of the eastern and western Arabian Sea.

Hypoxic zone	**East transect**			**West transect**			
Station	Stn 3	Stn 6	Stn 23	Stn 12	Stn15	Stn 17	Stn20
Biomass ml/100 m-3	60	2.3	6.7	12.3	3.4	32	7.1
Hydrozoa							
Siphonophorae					137	128	
Diphyes spp.	13653					512	
Medusae						128	
Trachy medusae							
Foraminifera				1969	137		
Globeginia					46		169
Gastopoda	245760		1280	394	46	384	
Pelecypoda			107		91		
Polychaeta	54613	5	1493	1772		512	155
Copepode nauplii		2			91	256	215
Juvinile copepods	13653	2	107			4096	585
CALANOIDA							
Acartia spp	2	1			46	1280	
Acartia erythrea			107				
Acartia amboinenensis							
Paracalanus sp.	81920	162	213	16			137
Acrocalanus sp	40960						46
Acrocalanus gibber							
Calanus sp		2		394	46	512	
Clausocalanus spp	327680		659			21.3333	
Cosmocalanus darwini		30	233	430	46		99
Canthocalanus pauper							
Calocalanus pavo	13653					128	
Calanoides carinatus					91	768	320
Undinula vulgaris	68267						
Eucalanus attenatus	40960		107	591		1024	320
Eucalanus subcrassus.	13653	3					96
Eucalanus mucronatus	13653						
Eucalanus elongatus					46		
Eucalanus spp	13653		533	591	366	4736	878
Rhincalanus cornuta			0	197	91		110

Rhincalanus nasutus			427	3742		2048	480
Lucicutia flavicornis		2					
Lucicutia ovalis			107		46	256	46
Lucicutia wolfendii			640				
Lucicutia clausii				1378	457	640	206
Lophothrix frontalis							46
Pleuromamma abdominalis		1280					
Pleuromamma indica	13653		3840	985	183	2176	1673
Pleuromamma gracilis			533	197			398
Pleuromamma xiphis							91
Metridia brevicauda							96
Scolecithricella spp		2	107	394	91		91
Scolecithrix ctenopus		2				512	
Scottocalanus helenae	40960						
Euaugaptilus sp					46		
Halioptilus					503		
Heterorabdus abbysalis			107		320	512	
Heterorabdus sp				197			379
Aetidena armature					46	128	46
MAcrosetella gracilis				197	137		142
Euchirella maxima							91
Euchirella venusta					46		
Euchirella spp					46		
Centropage furcatus				20		149	
Temora discaudata	13653						
Temora stylifera				20			
Calanopia sp			107	32	91		46
Labidocera sp.						128	
Eucheata concinna						128	
Eucheata marina	109227	2			46		
Euchaeta spp			213	394	274	512	123
Undeuchaeta spp			213				
MONSTRILLOIDAE							
Mormonilla phasma	54613	2	107	591	137		617
Mormonilla minor						768	46
POICILOSTOMATATOIDA							
Corycaeus catus	109227						
Coryceus specious						128	

Corycaeus sp.	395947	9	640	591	503	512	306
Faranula sp	68267						
Lubbockia squilamana					46		
Lubukia spp					46		
Oncea venusta	150187						
Oncea specious		59					
Oncaea sp.	655360		2453	7680	1554	5376	4457
Copilia mirabilis		2				128	
Sappharina gastrica				197			
Sappharina metallina						128	
CYCLOPIODA							
Oithona plumifera	68267		165	394	91		137
Oithona spinirostris	40960						
Oithona sp.	177493	122	320	1575	4480	2048	1339
Saphirella tropica							46
HARPACTICOIDA							
Clytemnestra scutellata						128	535
Clytemnestra rostrata	13653						
Microsetella rosea					46		78
Amphipoda	81920		107		137		
Ostracoda	409600		853	2560	594	512	2167
Decapoda							
Decapoda larvae	27307		320		366	128	187
Euphausiid			427		46		215
Lucifer typus	54613						
Chaetognatha							
Sagitta sp	218453	7	427	1969	1234	1280	686
Urochordata							
Oikiopleura sp.	505173	7	213				
Salp	13653			197	46		
Doliolum sp.	27307		107			256	32
Cephalopoda							78
Chordata							
Fish larvae	13653						46
Total org/100m3	4205229	1707	17270	29662	12937	33066.7	17736

Table: 5.4: Spatial composition of mesozooplankton in the suboxic waters of the eastern and western Arabian Sea.

Suboxic Zone	**East transect**			**West transect**			
Station	Stn 3	Stn 6	Stn 23	Stn 12	Stn15	Stn 17	Stn20
Biomass ml/100 m-3	0.23	1.47	1.30	5.50	0.53	2.00	1.20
Hydrozoa							
Siphonophorae			78				26
Diphyes	15333	6					
Medusae	2		39				26
Globeginia						973	122
Gastopoda		1	155	116		563	77
Pelecypoda	6						
Polychaeta	3833	283	110	116		51	38
Copepode nauplii	4	61	26			154	
Juvinile copepod	21	122	187	116		102	106
CALANOIDA							
Calanus sp					11		
Clausocalanus spp		309					
Calanoides carinatus			6	349	288	717	413
Eucalanus attenatus	4	62	148	582	32	154	92
Eucalanus elongatus		94					
Eucalanus spp	7673	411	175	1862	107	154	50
Rhincalanus cornuta			58	582	21		0
Rhincalanus nasutus	4		45	3491	11	358	256
Lucicutia clausii	4	51	6	116			58
Lucicutia flavicornis	48	179	39	582	11	307	208
Lucicutia ovalis		61					
Lucicutia wolfendii		73		465			
Lucicutia maxima						51	
Pleuromamma indica	30673	257	148	698			81
Pleuromamma gracilis		3	272				51
Pleuromamma xiphis			39				132
Pleuromama quadrata		91					4
Pleuromamma spp	7667				43		
Metridia sp						51	4
Metridia brevicauda				116			
Metridia venusta							106

Scolecithricella spp	1	1				51	26
Scottocalanus helenae	1						
Scotocalanus spp		13	13		11		
Scaphocalanus spp							26
Scaphocalanus elongatus							26
Augaptilus spp	15						
Euaugaptilus sp		4					
Heterorabdus abbysalis	1						
Heterorabdus subspiniformis				116			
Heterorabdus sp	53	34	90	233	11	102	4
Aetideus sp							51
Aegisthus mucronatus							51
Macrosetella gracilis			19	116			
Euchirella maxima		30					8
Euchirella venusta				116			
Euchirella spp		30		116			8
Calanopia sp	2	91					
Paracandacia simplex	6						
Eucheata marina	11500	213					
Eucheata wolfendii	7667				11		
Euchaeta spp	2	155		233	11	6246	
MONSTRILLIDAE							
Mormonilla phasma	35	503	194	931	11	410	262
Mormonilla minor					11		
POICILOSTOMATATOIDA							
Coryceus specious		30					
Corycaeus gibulus			19				
Corycaeus sp.	26838	95			11		55
Faranula sp	3833	30					
Lubukia spp	15						
Oncea venusta	34510	183					
Oncaea sp.	76715	1682	730	3491	107	1382	1603
Copilia mirabilis		1					
Copilia quadrata		1					
CYCLOPIODA							
Oithona sp1		129					
Oithona spinirostris							26
Oithona sp.	3840	311	291	349	11	358	283
Saphirella tropica			19				

HARPACTICOIDA							
Clytemnestra scutellata	2	35	58			51	26
Clytemnestra rostrata	3835						
Microsetella rosea		40					
Macrosetella gracilis			6	233			
Amphipoda	3833		6				
Ostracoda	6	459	291	116		154	126
Decapoda							
Decapoda larvae	3833	32	19	233			
Euphausiid spp.			6	116		51	26
Lucifer typus	11500						
Chaetognatha							
Sagitta sp	11506	296	136	698	64	154	166
Urochordata							
Oikiopleura sp.	46006	155					
Salp	3833	65			21	51	4
Doliolum sp.	3841	75	26	116			
Chordata							
Fish eggs	7667	1					
Fish larvae	3833	6	6			51	4
Ophophorus typus					11		
Total org/100m3	330004	6768	3461	16407	811	12698	4628

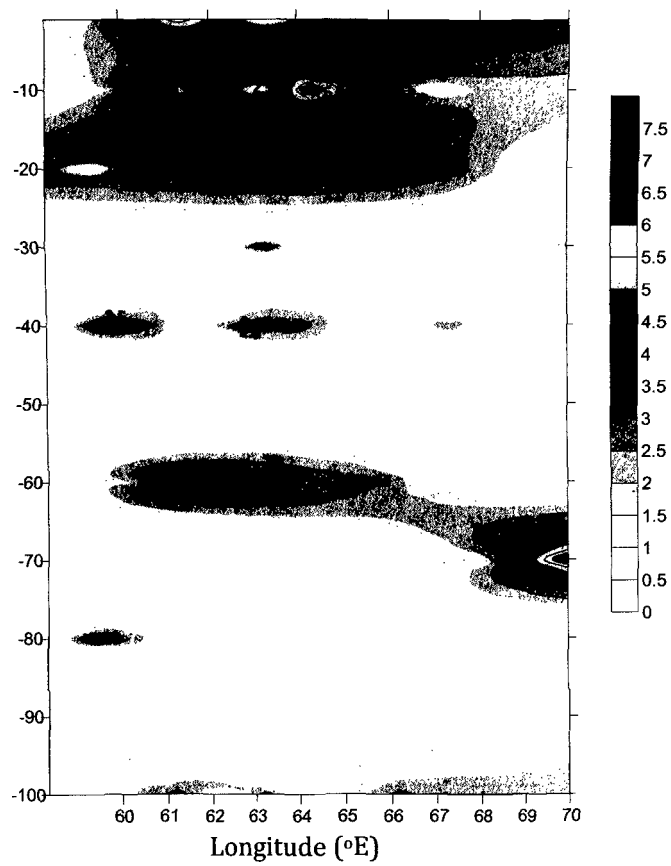


Fig. 5.2: Horizontal and vertical distribution of phytoplankton density (x105 l-1) in the euphotic zone of the Northern Arabian Sea.

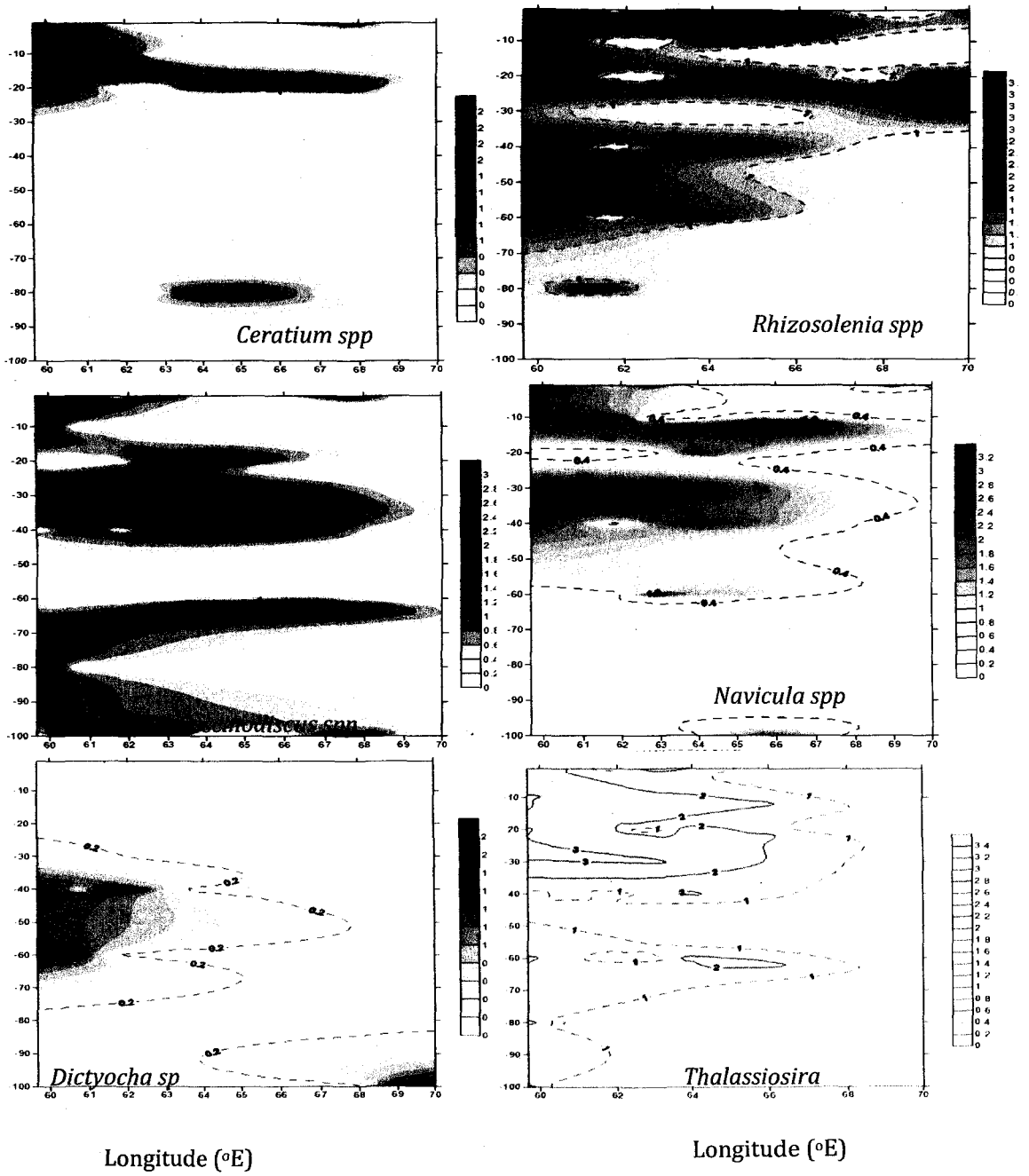


Fig. 5.3: Horizontal and vertical distribution of phytoplankton species (x105 l-1) in the euphotic zone of the Northern Arabian Sea.

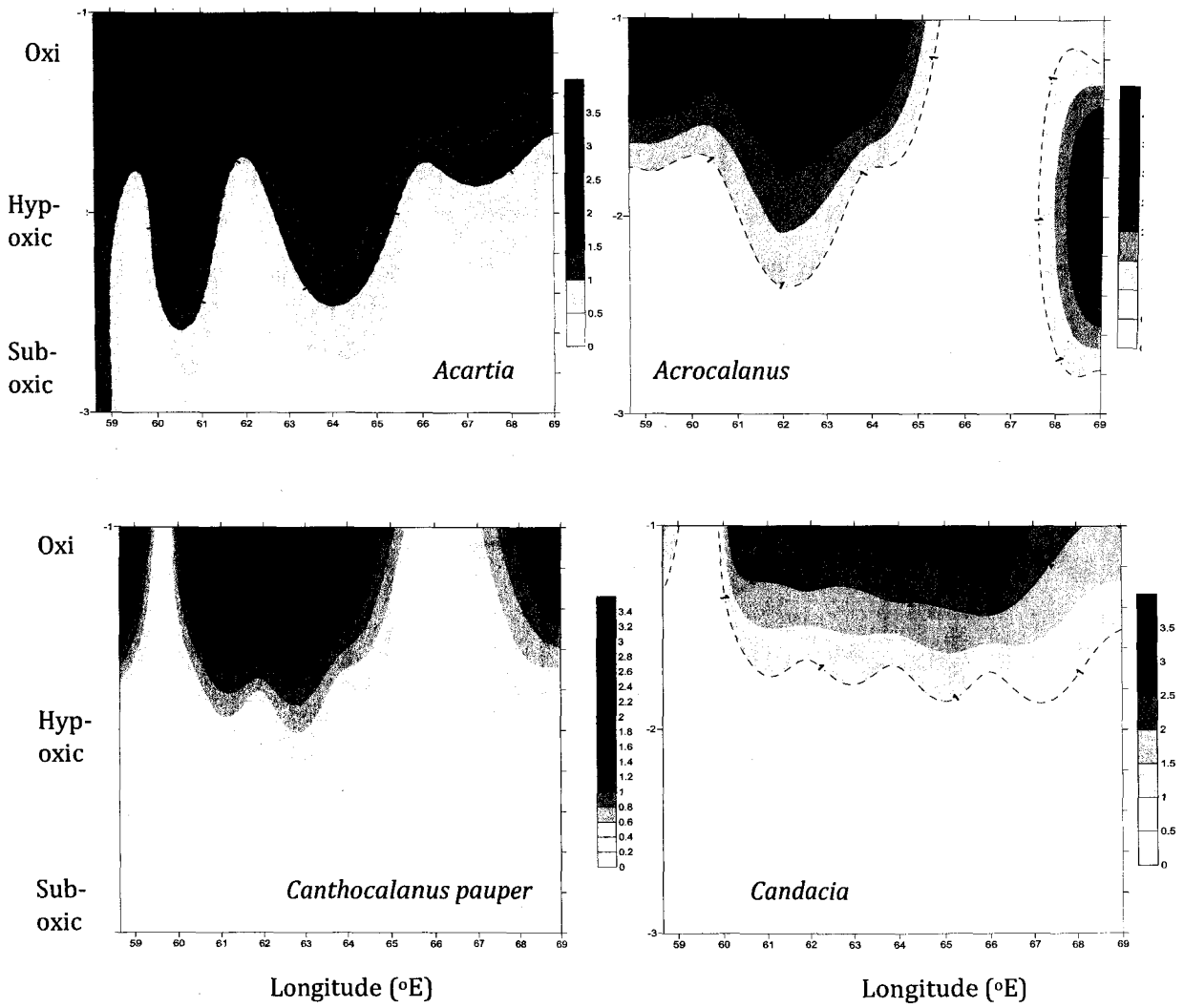


Fig. 5.4: Horizontal and vertical distribution of dominant copepod genera oxic region in the upper 100m depth.

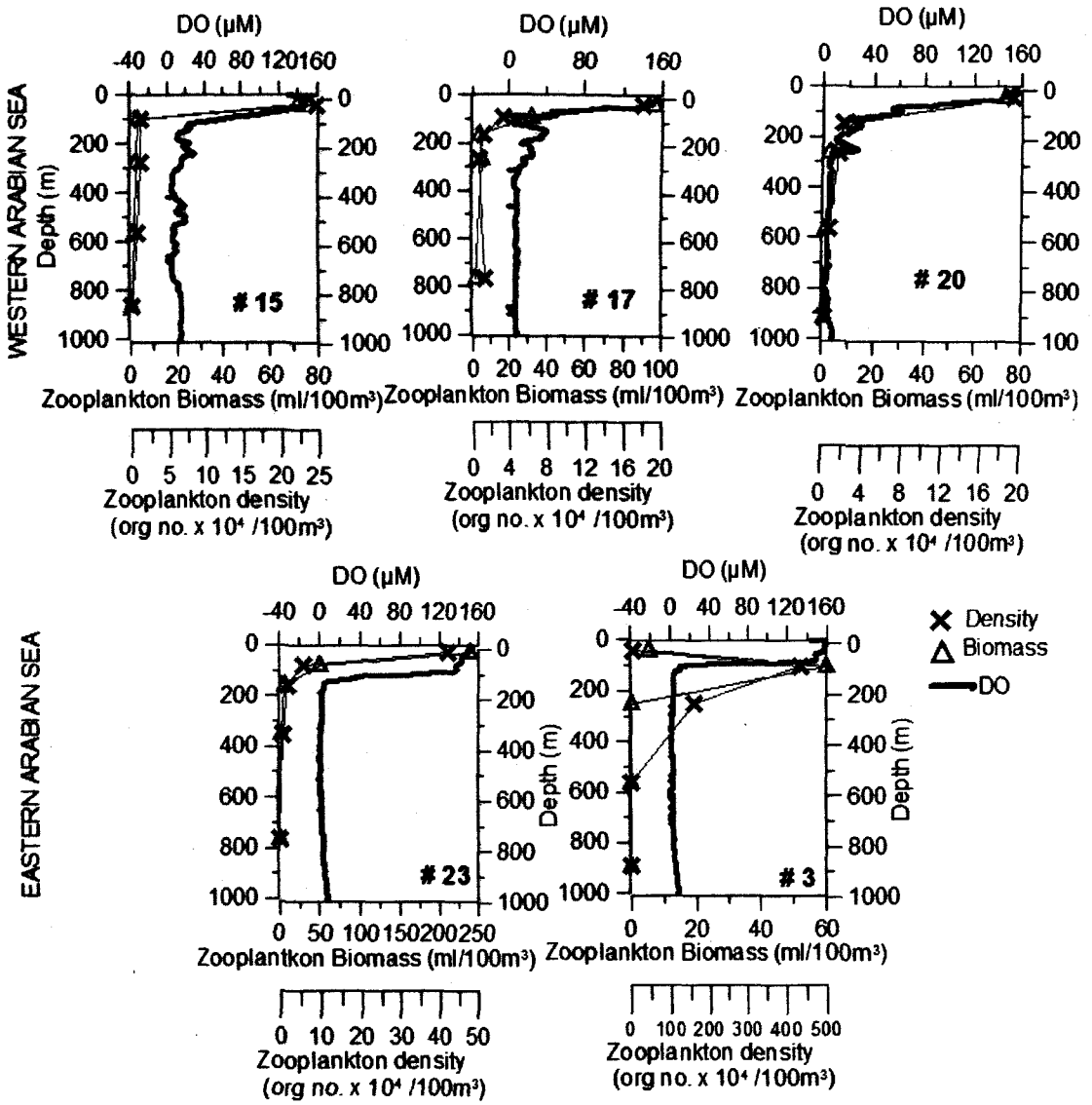


Fig. 5.5: Vertical variation in mesozooplankton biomass and abundance with dissolved oxygen gradient in the Arabian Sea.

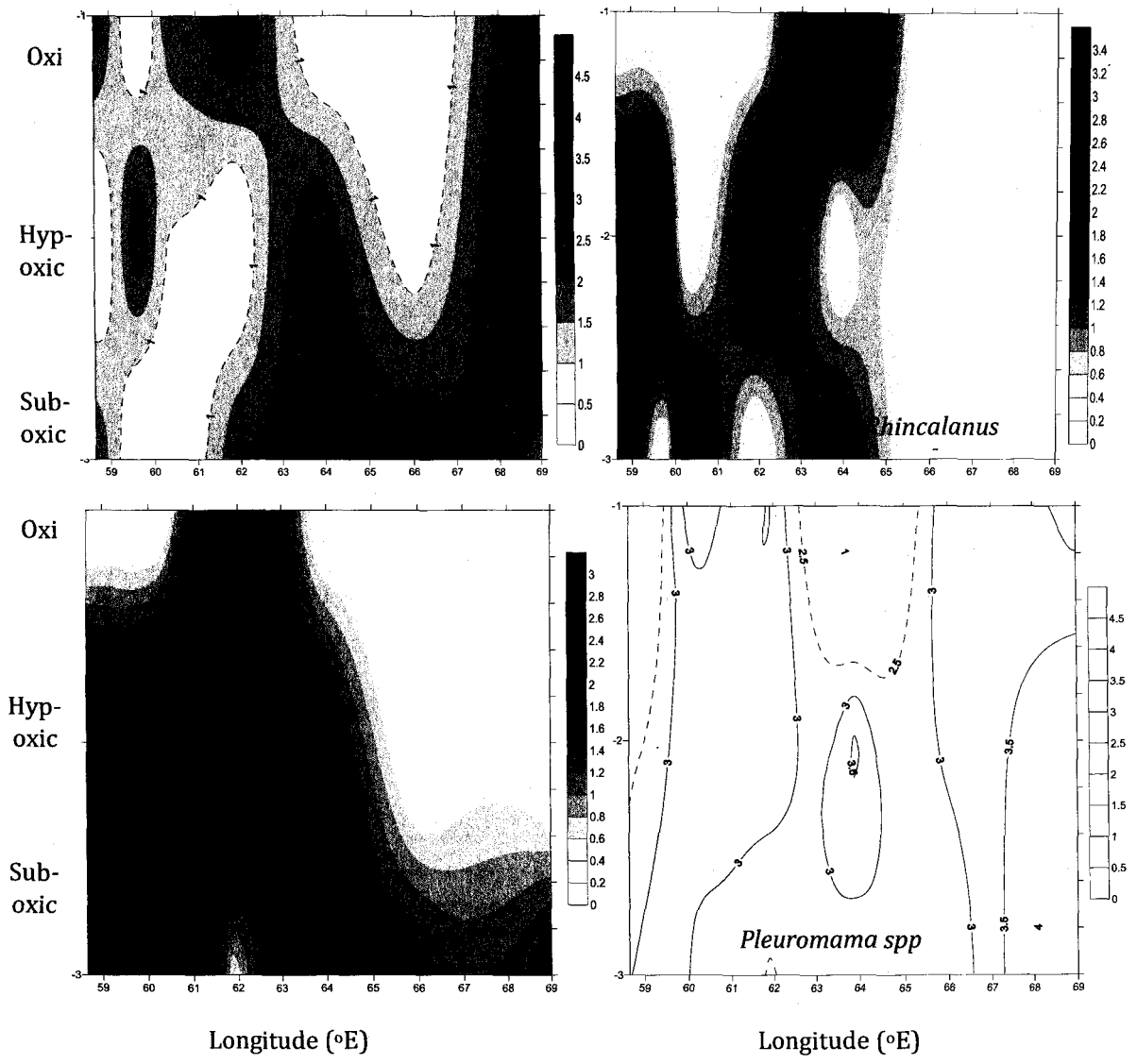


Fig. 5.6: Horizontal and vertical distribution of dominant copepod genera across an oxygen gradient.

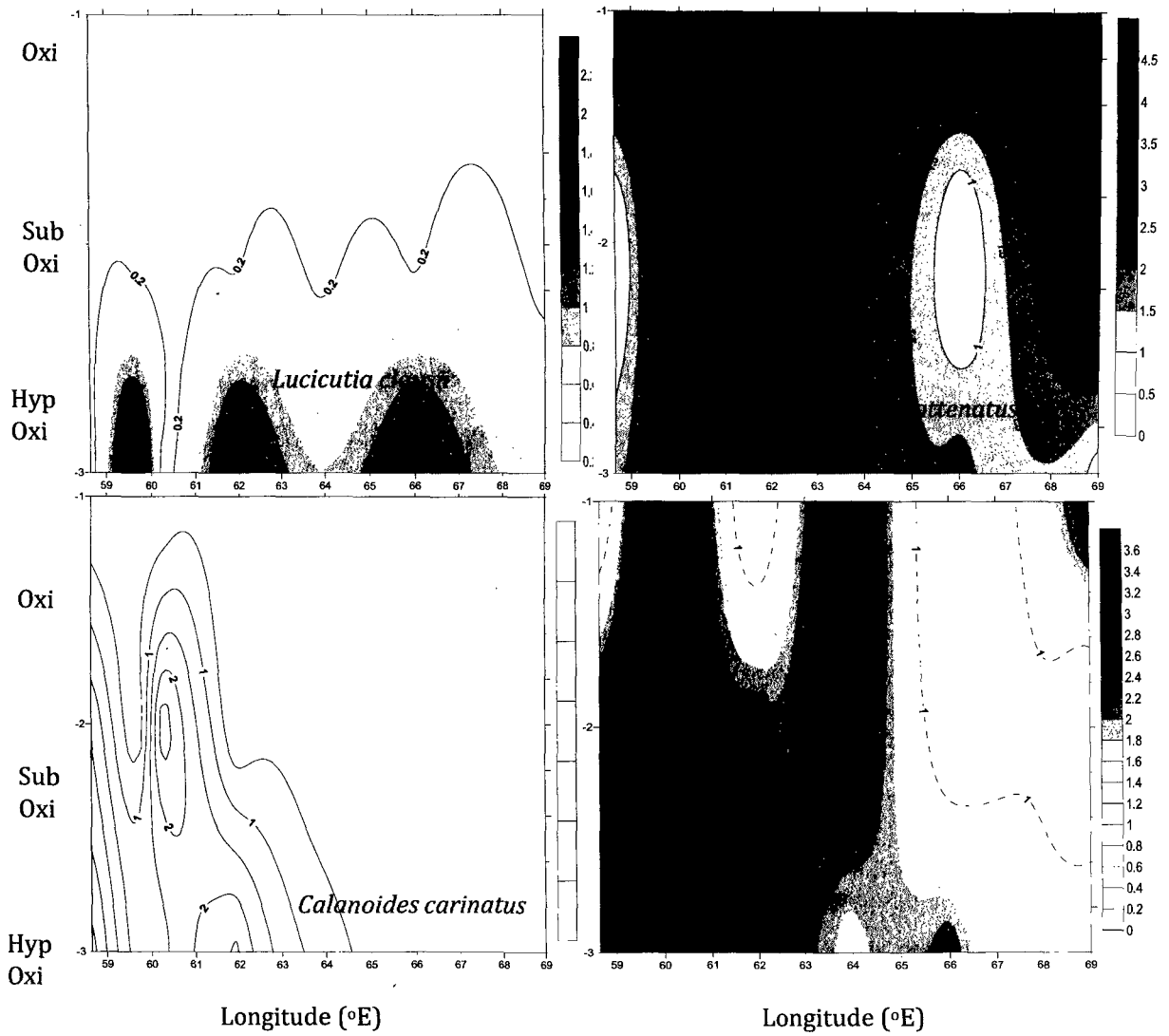


Fig. 5.6 (Cont.): Horizontal and vertical distribution of dominant copepod genera across an oxygen gradient.

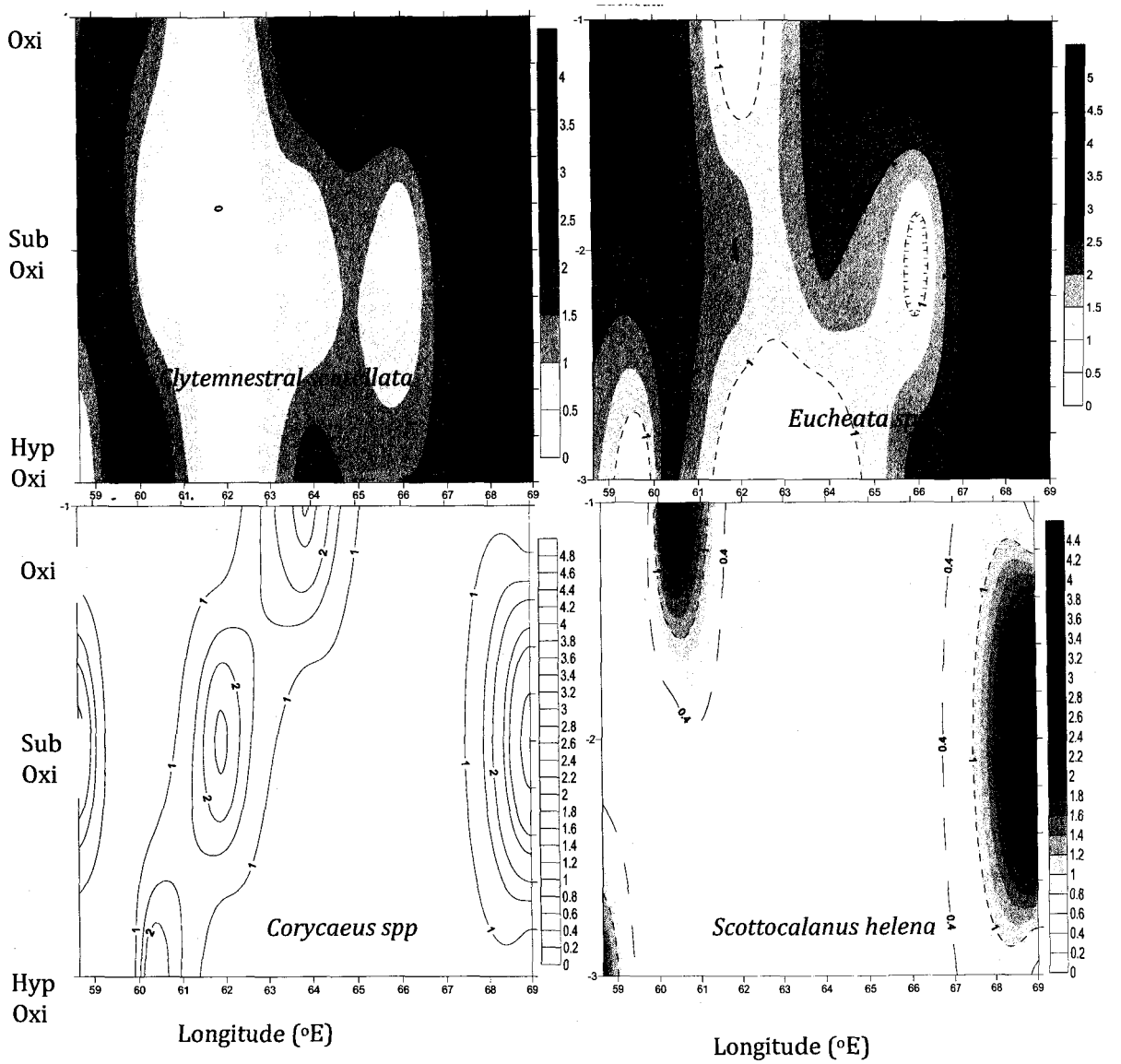


Fig. 5.6 (Cont.): Horizontal and vertical distribution of dominant copepod genera across an oxygen gradient.

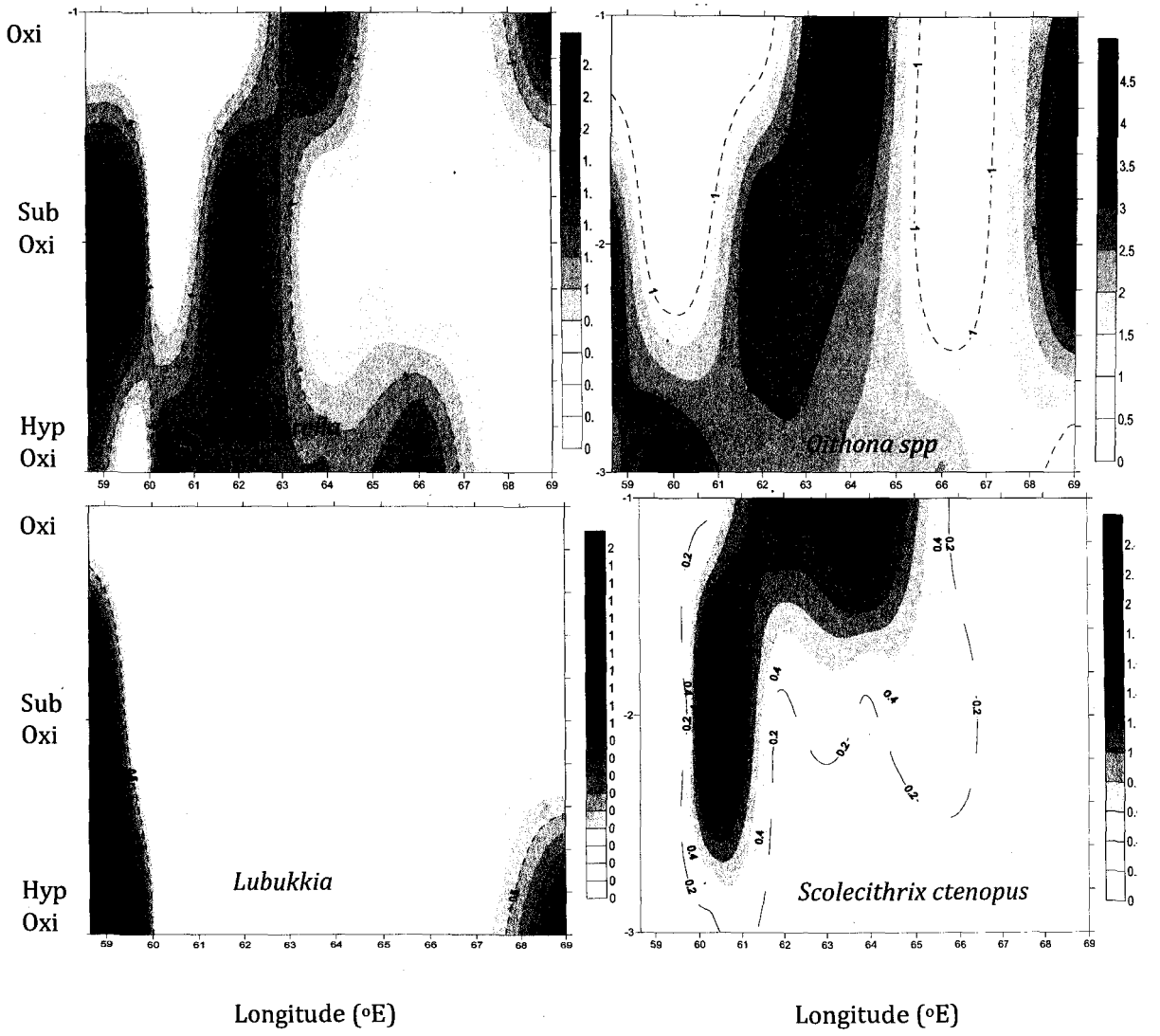


Fig. 5.6 (Cont.): Horizontal and vertical distribution of dominant copepod genera across an oxygen gradient.

CHAPTER 6

THE CONSORTIUM IN MARINE MICROPLANKTON OF THE NORTHERN ARABIAN SEA

The Arabian Sea is known for its great phytoplankton species diversity that changes seasonally. Observations on the symbiotic association epiphytic and endocrytic within and among phytoplankton, protozoan (ciliates in particular) and planktonic metazoans are scarce in the Arabian Sea. These associations were observed in the euphotic zone samples collected from the open waters of the Arabian sea water during late southwest monsoon (August-September'07) and associations recorded at the Candolim Time series transect (CaTS) are also presented. Some of which are first its kind record from the eastern Arabian Sea.

6.1: INTRODUCTION

The oceans are enormous bodies of water with numerous tiny microscopic plants (phytoplankton) and animals (zooplankton). Among the most abundant phytoplankton the diatoms and dinoflagellates are important primary producers in an aquatic system. They consist of a tiny blob of protoplasm enclosed in a transparent pill-box structure made of either silica or cellulose. Under favorable conditions, a single cell can reproduce at a very fast rate and at times one finds as many as a billion of them in a gallon of seawater. Most of these drifters rely on surface ocean currents in particular for their horizontal movement and distribution. Some of the phytoplankton has a great variety of unique patterns, forms and projection such as spines and bristles to the general cell shapes. These structures increases surface area of the plant body, which in turn reduces the sinking rate and help in absorption of water and nutrients directly from their environment and facilitate increased exposure to sunlight for photosynthesis to produce carbohydrates, proteins, fats, and oxygen. These products in turn are either consumed directly or indirectly by all other marine life forms from zooplankton to fishes and/or are subjected to infestation by parasites. And, in many cases symbiosis occur similar to terrestrial environment (e.g. in lichens; Taylor *et al.*, 1995). Cyanobacteria established symbioses with eukaryotes (e.g.

diatoms) at least 2.1 billion years (Lockhart *et al.* 1992; Raven 2002; Kopp *et al.* 2005). In case of diatoms, it is difficult to be sure that these associations are truly symbiotic, but it is certain that some forms are constantly found in association with other prokaryotes and eukaryotes. Symbioses are incredibly diverse as some exhibit ecto- and others endo - symbiotic associations. Familiar examples include, *Ornithocercus* spp and cyanobacteria (Norris, 1967; Taylor, 1982; Gordon *et al.*, 1994; Jyothibabu *et al.*, 2006) wherein latter ones provides nitrogen for dinoflagellate. Similarly, *Krytoperidinium foliaceum* and *Peridinium balticum* reveal intracellular symbiosis (Tomas and Cox, 1973; Jeffery and Vesk, 1976).

Association has significance in regions where nitrogen limitation is prevalent. But, the ecological significance of this association is still not much known. In the northern Indian Ocean, cyanobacteria are known to play a major role in the pelagic food web especially when the upper euphotic zone is stratified and nitrate concentration is very low (<0.01μM) condition, which prevail in the AS during some season (Burkill *et al.*, 1992; Krishnaswamy and Nair, 1996) particularly fall and spring. Symbiotic associations in the Indian ocean has been known since the International Indian Ocean Expedition (IIOE) during which few of the cyanobacterial symbionts were isolated and cultured (Norris, 1967) . Even though biogeochemistry of the Arabian Sea is being studied since John Murray Expedition (in 1930's onboard *Mabahiss*), information on seasonality, ecological significance of these association is very little known. Recent work on phytoplankton from the Arabian Sea (Sawant and Madhupratap, 1996; Parab *et al.*, 2006) and Bay of Bengal (Paul *et al.*, 2007) have no mention of such symbiotic associations. It is quite possible that such associations were previously overlooked. The objective of this work is to document consortia observed in the northern Arabian Sea (late SWM in particular) in response to the prevailing physico-chemical and other biological parameters at the time of their occurrences.

6.2: MATERIALS AND METHODS

The monsoon cycle is a major factor, which influences the hydrographic features of the Arabian Sea. Accordingly, months of the years has been divided into four seasons *viz.* southwest monsoon (June-September), fall intermonsoon (October-November), northeast monsoon

(December-March) and spring intermonsoon (April-May). Consortia observed in samples collected from the Arabian Sea during a cruise aboard *Roger Revelle* (RR) between 23 August'07 and 16 September'07 (Fig. 6.1) are presented. Cryptic association(s) found during year-round sampling (2005-06; except during peak monsoon months, June-August, when sea remains rough) made at the CaTS transect situated perpendicular to the coast of off-Goa (Fig. 6.1) are also described. All water samples were obtained either with a CTD – rosette (during RR cruise) or hydrocast (CaTS site) equipped with 5L Niskin samplers (General Oceanics, Miami FL, USA). Temperature-salinity (and fluoroscence) data was obtained from Sea Bird CTD and at times reversing thermometers were used to record subsurface temperatures at the coastal transect - CaTS.

Routine chemical analyses of water samples for dissolved oxygen and nutrients were performed following standard procedures (SCOR, 1996). Dissolved oxygen was analyzed following the Winkler titrimetric procedure using an automated system built and supplied by the Scripps Institution of Oceanography/Ocean Data Facility (SIO/ODF) group. Nutrients were measured with a SKALAR autoanalyzer.

Biological parameters (phytoplankton, synechococcus, het. nanoflagllates, bacteria) were collected and processed following JGOFS Protocols (UNESCO, 1994; Grasshoff, 1983; SCOR, 1996). Samples for chlorophyll *a*, *Synechococcus*, het. nanoflagellates, bacteria were kept appropriately at low temperature (icebox / refrigerator / liquid nitrogen) soon after collection to minimize grazing and pigment losses. Protocol for chlorophyll and phytoplankton is already given in details in chapter 3.The protocol for TBC and HNF is given below.

6.2.1: BACTERIAL ABUNDANCE (TBC)

Twenty ml water samples were fixed with formaldehyde (2% final concentration) and refrigerated in the laboratory. Sub-samples of 2 ml were stained with 4,6-diamidino-2-phenylindole (DAPI) and filtered onto a black 0.2 µm pore size Nucleopore filters (Porter and Feig, 1980) and slides prepared. Bacteria were enumerated using UV excitation. A minimum of

20 fields were counted for each sample at 1000X in an Olympus BH2 epifluorescence microscope and cell numbers calculated by following Parsons *et al.* (1984).

6.2.2: HETEROTROPHIC NANOFLAGELLATE (HNF)

A 50 ml of water sample was fixed in 2% glutaraldehyde (final concentration). DAPI and Proflavin were added to 5-10ml sub-samples to a final concentration of 5 $\mu g\ ml^{-1}$ each and allowed to stain for 5 minutes (Hass, 1982; Booth, 1993) and filtered through 0.8μm black Nuclepore (Nuclepore, USA) filters (Sherr and Sherr, 1983; Booth, 1993). Slides were prepared and held at 5°C in a darkened box until taken up for epifluorescence microscopy. Only unbroken well-defined individuals were counted and there biovolumes determined.

On cruise, chlorophyll *a* samples were analyzed immediately. All slides for bacterial and HNF counts were prepared onboard. A satellite image of chlorophyll *a* concentration, sea surface salinity (SSS), photo synthetically available radiation (PAR), Dissolved and particulate carbon concentration for the study period were derived from the SeaWIFS sensor.

6.3: RESULTS

6.3.1: EPIPHYTIC ASSOCIATIONS

Warm waters in particular are known to exhibit a great variety of associations in the oligotrophic environments (autotrophs-autotophs or autotrophs – hetrotrophs) particularly in the tropical waters (Gaines and Elbrachter, 1987; Guillard and Kilham, 1977; Tayor, 1982, 1990; Gordon *et al.*, 1994) wherein one or both partners are benefited. Generally, associations are known to have importance in a region where nitrogen limitation occur. Such conditions prevails in the AS particularly during spring and fall intermonsoon period during which surface waters nitrate concentration remains undetectable (DeSouza *et al.*, 1996). Similar, conditions also prevail in the Bay of Bengal (BOB) as well (Sardessai *et al.*, 2007) even though fresh water input into the Bay is far more than in the AS (Subrahmanyam, 1993). Resultant stratification in both these region, though processes are different, lead to low production including summer monsoon

in the BOB (Bhattathiri *et al.*, 1996; Madhupratap *et al.*, 2001; Gauns *et al.*, 2005). It is still unclear whether this two process of stratification lead to different consortia and if so, do they exhibit any seasonality? Recently, cyanobacterial associations from the BOB comes from the work of Jyothibabu *et al.* (2006) highlighting dinoflagellates (*Ornithocercus spp.*) cyanobacteria association. Previous work on these dinoflagellates has been reported from the Gulf of Aqaba by Gordon *et al.* (1994). However, extent of benefits can only be studied through experimental studies that are lacking. Work of Taylor (1982) from tropic and subtropical region report 40 such associations. Other consortia work include of Kimor *et al.* (1992) from the Red Sea.

Consortia in the present study were seen in both pennate and centric forms of diatoms. The complex shapes and structure in phytoplankton community have been hypothesized to serve many ecological roles, including aids to flotation, and/or protection from predators. Besides, complex morphology also offers a structural substrate for a variety of epiphytes (see below). Generally, in such association, host need not necessarily be diatoms even copepods (harpacticoid) are known to form host (Kimor *et al.*, 1992).

6.3.1 (a): *Chaetoceros coarctatus* (CC) associated with *Vorticella oceanica (plate 6A).*

Chaetoceros coarctatum is an oceanic warm water marine chain forming species (Karawada, 1965). This branching centric diatom first described by Lauder (Lauder, 1864) has cylindrical/elliptical cells forming robust long, straight, rigid chains. Setae arising within the chain margin are of 3-4 different types, some are short and straight and others tightly curved. Few are very thick with moderately to heavily silicified. This 30-50μm size cells (Hernández-Becerril, 1991; Hustedt, 1930) are with numerous small chloroplasts found both in the cell body and in the setae. These morphological features together with other outgrowths increase the surface for floatation.

Chaetoceros valves are typically found with sessile protozoan species of *Vorticella* (species *oceanica*) attached with its characteristic long contractile stalk (Plate 6A), sometimes in large numbers. In the present study, this association was found at the surface oxic (3.4 ml/L), low temperature (24.9°C), saline (35.7psu) nutrient rich waters (ca. 10.8μM NO_3; 0.75μM PO_4 and

2.4μM SiO_4) of stn 17. Some of the chambers of *Chaetoceros* (*Ch.*) were with as many as 4 Vorticella (~30–40μm wide). Though called oceanic, similar associations were also found at the CATs location in the month of January of NEM characterized by low temperature (26°C), high salinity (35.5) and favorable *NO3* concentration (0.52 μM). Contribution of CC to the total phytoplankton cell abundance at stn 17 was much less (~1.3%) than those reported by Nagasawa (1906) from the Otsuchi Bay (ca. 73% of the total cell number). Observation of their consortia at the surface was previously recorded by Karawada (1965). This association is being described as epibiosis by Lincoln *et al.*, (1982). PROTOZOAN'S (VORTICELLA) are quite prolific in both cold Antarctic waters as well as tropical, subtropical and temperate and is found attach to all kinds of substrates. A record of *Chaetoceros* association with *Vorticella oceanica* is being mentioned in the work of Zacharias (1906), Kokubo (1960); Yamaji (1984), Fujioka (1990) and Aleem (1979) from different parts of the world ocean. Similar association was previously recorded in the Gulf of Aqaba, Red Sea (Kimor *et al.*, 1992) and in the eastern Mediterranean Sea (Zismann *et al.*, 1975; Kimor, 1983).

On the other hand, some species of *Chaetoceros* (eg. *decipiens*) are also known to host pennate naviculoid diatoms as epiphytes in the coastal waters of Southern California (Kimor *et al.*, 1992). Apart from *Chaetoceros C*, *Vorticella* in tropical waters are also found attached to *Thalassiosirapunctigera*(http://serc.si.edu/labs/phytoplankton/guide/addtl_collections/Belize%202/tpuncvort.aspx) and tintinnid (*Eutintinnus inquilinus* Mull; see Kimor *et al.*, 1992).

6.3.1 (b): *Rhizosolenia alata* Brightwell *f. indica* (RA) associated with colony of *Zoothamnium sp.* (plate 6B)

The diatom, *Rhizosolenia. alata f. indica*, is a tropical warm water form found both in the coastal and in oceanic waters. This hair type long narrow cells are cylindrical and straight with tube like end contains numerous chromatophores and protoplasmic streaming, which is conspicuous only in this genus. Each cell (ca.7-70μm) has curved oblique process where apex of the neighboring cell fits. This characteristic structure help *Rhizosolenia* to maintain them in floating condition. Unlike, *Chaetoceros coarctatus* associated with solitary *Vorticella* as described earlier, *Rhizosolenia* sp was found associated with a colony of *Zoothamnium* sp.

attached to the host cell with one connection and, each bunch was with 8-28 individual zooids (Plate-6B). *Zoothamnium* is a genus of the group sessiline peritrich ciliates, forms branching colonies.The colonial zooids share continuous myonemes and entire colony contracts. The colony can grow upto 2 to 3 mm height, which are visible to naked eye. They are 50-120 micron size and around the the world live in the sea and brackish water, but can also occur in fresh waters.

Some species of the *Rhizosolenia* are also known to encompass *Richelia intracellularis* (a symbiotic species; see below), a N_2-fixing endosymbiotic diazotrophic cyanobacteria living inside the cell (Carpenter *et al.*, 1999), which can be easily identified under fluoroscence microscope. However, we did not observed such consortia in the present study. Possibly, due to use of Lugol's Iodine as a fixative/preservative, which probably masked symbiont auto fluoroscence. This endosmbiont *Richelia* are commonly found in the tropical Pacific Ocean areas (Venrick, 1974; Villareal, 1992) living in *Rhizosolenia* (and *Coscinodiscus*) apart from *Hemiaulus* (See below), which at times forms blooms in the oligotrophic ocean (Carpenter, 2002) thereby primary production was substantially elevated (Carpenter *et al.*, 1999).

In the present study, association of *Rhizosolenia alata* and *Zoothamnium* sp. was found at the sub-surface depth (20m) of stn 17. This species ~11% of the total phytoplankton cell abundance (6363 l^{-1}). Mixed layer depth being relatively deeper (~50m) at this location, nutrient concentration was higher (NO_3= 10.8µM, as mentioned in *Chaetoceros* and *Vorticella consortia* mentioned above), high saline (35.7 psu) and cooler (24.8°C). Work of Devassy (1974) record bloom of RA (chl a: 123.47 mg m^{-3}) near the coast of Mangalore (west coast of India) associated with cooler, saline and nutrient rich waters (1.78µM of Nitrate and 2.4µM PO_4) in the month of August. Interestingly, even though stn 17 was characterized by wide diversity of *Rhizosolenia* species (*imbricate, hebetate, robusta, setigera, shrubsolei, sloterforthi, styliformis* and few unidentified species), *Vorticella* association was restricted only in *R. alata*. This is one of the grey areas that need investigation to understand chemistry between the two.

6.3. (c): *Leptocylindrus mediterraneus* (LM) associated with flagellates *Solenicola setigera* (SS) (Plate 6C)

This centric diatom *Leptocylindrus mediterraneus* (=*Dactyliosolen meditterraneus) is* a cosmopolitan form recorded from Antarctic to the Arctic, but never (or seldom) occur as a dominant species (Hasle, 1976). This genus has weakly silicified cylindrical cells mostly in chain form (like the ones found in the present study, Plate 3) but at times single cells were also found. Most of the cells were devoid of photosynthetic pigments as pointed out in the Kimor's study (Kimor *et al.*, 1992) and intercalary bands were with associated epiphyte *Solenicola setigera.* This epiphytes are flagellated colonial protozoan (ca 6-20μm in diameter and 91-156μm in length) with one flagellum generally found attached to submerged objects including diatoms often forming aggregates. The LM-RS consortium is common and wide-spread in the Pacific Ocean between 41°N and 34°S and in the oligotrophic waters of the open-ocean, and tend to appear near the deep chlorophyll maxima with a low (<20 frustules l^{-1}; Gomez Fernando, 2007; http://cat.inist.fr/?aModele=afficheN&cpsidt=20183036) to high abundance (ca 8000 frustules l^{-1}) in more eutrophic waters. Kimor *et al.* (1992) reports LM-RS association as deep as 250m in the Gulf of Aqaba. But, in the present study, association was found in near surface waters (5mt) of stn 19 contributing roughly 5% of the total phytoplankton cell abundance (7400 L^{-1}). Upper water column of this region was representative of typical upwelled advected waters (dissolved oxygen=3.3 ml/L; SST=26.7°C, SSS= 36 psu; NO_3=0.97μM; PO_4=0.27μM; SiO_4=0.78μM). Some of the specimens of LM were free-living and without any associations. Interestingly, the frustules used by epiphyte as a substrate exhibited an abnormal morphology suggesting that the protozoan may be able to control the growth of the frustules (Kimor *et al.*, 1992).

6.3.1(d): *Trichodesmium erythraeum* (TE) bloom associated with unidentified globular/elliptical cells (Plate 6D)

Some phytoplankton such as the blue green alga have ability to fix large amount of atmospheric nitrogen particularly *Trichodesmium* (Capone *et al.* 1997; Codispoti, 1995). This together with *Richelia* (blue green alga; see below) supply about 25% of the total N demand in the water column (Carpenter *et al.*, 1999). They can be an important source of new-N to the

euphotic zone of nutrient poor water (Fogg, 1982) such as in tropical waters. Thus, they are able to grow and make new production in places where nitrate and ammonia are lacking and in making possibility of exporting carbon even in oligotrophic environment (Abell *et al.*, 2000, Marchal *et al.*, 1996). Thereby playing an important role in the nitrogen budget of the sea. Presence of this blooms are an indication of calm, low nutrient waters (Capone *et al.*, 1998). This filamentous cyanobacteria known to form aggregates (trichomes) supporting internal O_2-reduced microzones where N_2 fixation is thought to be concentrated (Bautista, 1985; Bryceson and Fay, 1981; Carpenter and Price, 1976; Paerl and Bland, 1982).

Though patchy, the non-toxic blooms of *Trichodesmium* are a marked phenomenon that occurs every year in the Arabian Sea from February to April (pre-monsoon period; Devassy *et al.*, 1978; Qasim, 1970). Ideally, fair weather season characterized by calm sea with high sea surface temperature (27-31.8°C), high salinity (35-36 psu), low wind (15-20 km h^{-1}), low currents (10-15cms sec^{-1}) and high sunshine period (8-11hrs) and stable top layer are reported to be favorable conditions required for their blooms. Sprinkled saw dust appearance of this is due to their confinement in the surface water. High Chl *a*, high POC and high phytoplankton diversity (39-44 species) and zooplankton community is found associated with these blooms (Devassy *et al.*, 1978). Senescent stage of the blooms is known to enrich water by releasing phosphorus and nitrogen (particularly NH_4-N) supporting succession in some diatoms such as *Nitzchia closterium, N. seriata, Chaetoceros* spp., etc.

This association of *Trichodesmium erythraeum* and unidentified bulbous cells (some appeared as dividing cells; Plate 6D) was recorded in the month of April'07 at stn G2 of CaTS transects. Bloom was intense roughly forming 83% of the total phytoplankton community ($3.4x10^6$ L^{-1}). Other forms were represented by few genera of diatoms (8) and dinoflagellates (2). Further, intensification of bloom resulted trichos cell abundance as high as 4.8×10^7 cells l^{-1} forming ~91% of the total phytoplankton community; chl *a* concentration increased to 128 mg m^{-3}. Consequently, both dinoflagellate and diatom community dwindle particularly former group. Diatoms were represented by only *Chaetoceros curvisetus*, *Leptocylindrus minimus* and *Nitzchia closterium* (and no dinoflagellates were seen). Concurrently, cell abundance of the associated unidentified epiphytic cells also increased roughly by 18 fold (0.06×10^4 L^{-1} to 1.14 x

10^6 L^{-1}). As expected, this association was in stratified high saline (36psu) waters that were relatively warm (SST, 30°C) and low in nutrient (NO_3=1.25µM; PO_4=0.9µM; SiO_4=9.2µM). Unfortunately, their autofluoroscence could not be tested since samples were preserved in Acid Lugol's solution. Further, associated with this intense bloom large abundance of bacterial population was observed under a microscope (Plate 6B) indicating senescent phase of the bloom. Associations of trichomes with bacteria's are well documented in work of Siddiqui *et al.* (1992), Nausch (1996), and Mulholland (2007).

6.3.1(e): *Coscinodiscus gigas* (CG) associated with protozoan like cells (possibly ciliate) (Plate 6E)

This centric diatom is a rare species in the Indian Ocean. Culture of this species was recently studied by Yogamoorthi (2007) to understand effect of UV-B radiation on its photosynthetic oxygen release. A consortium between *C. gigas* and the ciliate was previously recorded in the shelf waters of north and north-west Australia by Hallegraeff and Jeffrey (1984). In the present study, association of this diatom with epiphyte (Plate 5) was found during oligotrophic period (late March) in the coastal water of CATs location (stn G5). Water column was characterized by warm SST (29.5°C), high saline (35.5psu) and low in NO_3 and PO_4 concentration (~0.5µM). However, silicate concentration was not limiting (10.9µM). This consortia was associated with diverse phytoplankton community (26 species) and the most dominant ones that together contributed >50% were *Gunardiella striata, Thallasionema nitzschiodes, Thallasiosira subtilis, Skeletonema costatum, Nitzchia closterium* and *Chaetoceros curvisetus*.

6.3.1(f): *Pseudonitzchia seriata with unidentified forms* (Plate 6F)

Geographically, *Pseudonitzschia* is a commonly distributed genus (Hasle, 1965, 1972) restricted to marine plankton found both along the coasts and in open seas. Long and narrow cells of this genus form stepped chain united by shorter or longer overlap of valve ends and are characterized by fine striations. Some species of *Pseudonitzhia* (e.g. *P. multiseriata*) are known

to produce neurotoxins, domoic acid particularly during stationary stage (Lindholm *et al.*, 1994; Bates *et al.*, 1989; Fryxell *et al.*, 1997) and are generally associated with amnesic shellfish poisoning found in the coastal waters. At times bloom of this species causes mass fish mortality together with other toxic dinoflagellate species (*Pseudonitzchia seriata, Alexandrium minutum, Gymnodinium* sp., *Prorocentrum micans, Dinophysis rotundata, Heterosigma cf. akashiwo Gymnodinium cf. mikimotoi (=Karenia mikimotoi), Prorocentrum minimum, Noctiluca scintillans)*. In the present study, consortia was found in the surface coastal waters during winter when SST was low (~26°C), SSS was high (35.5psu) and in relatively high nitrate condition (1.1μM). Phytoplankton biomass (Chl *a*) was also moderately high (3 mg m^{-3}) in the region. Previous work of Devassy and Goes (1989) reported this species in the estuarine waters of Mandovi-Zuari in low saline (27.3 psu) and high nutrient condition (>4μm NO_3 and >1μM PO_4).

6.3.1(g): Ornithocercus *quadrates with Synechococcus carcerarius* (Plate 6G)

The dinoflagellates are a large group of flagellated protist and nearly half of all dinoflagellates are non-photosynthetic. In general, dinoflagellates form an important part of the aquatic food chain. Blooms of these groups can result in a visible coloration of the water. Genus *Ornithocercus* (and *Histoneis)* is generally known to be distributed in the deeper waters of tropical and subtropical seas (Gaines and Elbrachter, 1987; Taylor, 1987) and are devoid of any photosynthetic pigments, feeding by osmotrophy (Droop, 1974). Therefore, in many instances, these dinoflagellates host clusters of cyanobacterial cells in their body known as 'phaeosomes' (Norris, 1967; Taylor, 1982). But, the ecological significance of this symbiont has remained mysterious for many years. Recent literature suggests that the association has significance in regions where nitrogen limitation is prevalent. These regions are active sites for cyanobacteria to proliferate (Johnsen and Sieburth, 1979). In the northern Indian Ocean, oligotrophic condition prevails during spring and fall intermonsoon during which picoplankton dominate when very low concentration of nitrate nutrient prevails (Burkill *et al.*, 1993). In such oligotrophic environment, heterotrophic dinoflagellates provide favorable micro-environments to cyanobacteria for the efficient fixing of molecular nitrogen (Gordon *et al.*, 1994). However, occurrence of association at near surface oxic water (3.5 ml L^{-1}) could retard the process of nitrogen fixation by

inactivating the enzyme involved in nitrogen fixation (nitrogenase). Thus, cyanobacteria being inside the body of heterotrophic dinoflagellate may be an advantageous to get nitrogenase activity going-on more efficiently in reduced oxygen concentration then their relatives in the surrounding water. Further, respiration activity of both host and symbiont also could further enhance the process of reduced micro-environment. That is, cyanobacteria (phaeosomes) are shown to exude a large proportion of their fixed carbon to the host there-by accelerate oxygen consumption in their microenvironment. Thus, 'phaeosomes' gets advantage in nitrogen fixation by remaining inside the body of heterotrophic dinoflagellates and has special significance in oligotrophic environments where high oxygen tension is normally antagonistic to nitrogenase activity. To overcome this constrain of dissolved oxygen concentration some cyanobacteria possess 'heterocysts' that enable them to create low oxygen micro-environments (Wolk, 1982). Although the efficiency of fixation in this form may be less, but still non-colonial, non-heterocystous cyanobacteria are also able to fix nitrogen in highly oxygenated, oligotrophic waters (Kallas *et al.*, 1983., Paerl *et al.*, 1989) where this cyanobacteria form low oxygen tension at the centre of their colonies to enable nitrogenase activity (see Kallas *et al.*, 1983).

During the present study, dinoflagellates *Ornithocercus quadratus* were found with cyanobacterial (*Synechococcus*/ *Synechocystis*) symbiont (Plate 7). This association was found in surface waters of stn 9 that was characterized by low temperature (27.5°C), high saline (36 psu) and low nutrient condition (NO_3=0.04μM; PO_4=0.16 μM; SiO_4=0.71 μM). Occurrence of these consortia in surface waters was previously reported by Taylor (1987), Gaines and Elbrachter (1987) and Gordon *et al.*, (1994). Prevailing oligotrophic condition in the upper water column (<60 m water column had <0.01 μm NO_3), possibly favored the proliferation of cyanobacteria cells. *Synechococcus* counts as high as ~20 x 10^6 ml^{-1} were recorded at some of the stations (Gauns *et al.*, submitted to Marine Biology). In the present consortia, the cyanobacterial population were found located externally between the upper and lower cingular list in *Ornithocercus* (unlike in *Histioneis* where symbiont live within a chamber on the girdle floor). Under epifluoroscence microscope, orange or yellow fluorescence of phaeosomes' contrasts with the green fluorescence of heterotrophic dinoflagellates (Gordon *et al.*, 1994), which was not attempted due to preservative constrain mentioned above. Other dinoflagellates showing this symbiont include *O. magnificus, O. heteroporus, O. quadratus, O. steinii, O. thumi* and

Histioneis hyaline were recorded from the Bay of Bengal by Jyothibabu *et al.* (2006). These authors found associations at subsurface depth (75m), maximum during spring intermonsoon (1747 individuals; 96% showed association) followed by winter (964; 15%).

6.3.1(h) : *Hemiaulus membranaceus* with *Richelia intracellularies* (Plate 6H)

Hemiaulus is a centric chain forming diatom roughly 15-35μm in cell length commonly found in warm oceanic waters. Other common species of this genus include *membranaceus, hauckii, indicus, and sinensis.* Former species is known to occur very abundantly in the North-eastern Mediterranean Coast of Turkey during summer period and at times reaching as high as 28,480 cells l^{-1} (Polat and Isik, 2002). In our waters most common species is *H. hauckii* that occurs for most part of year at times contributing as much as 19% (7750 cells L^{-1}) of the total phytoplankton community. On the other hand, *H. membranaceus* a less common species was found at the surface in the month of August at station G5 (and G3) roughly forming 4% (2808 cell L^{-1}) to 10% (1560 cells L^{-1}) of the total phytoplankton community. Consortia of this species with *Richelia intracellularies* was observed at 9mt water depth of stn G5 (Plate 8). Waters of this depth were characterized by low temperature of 23°C, high saline (35.5psu), low oxygenated (0.6ml/L) and rich in nutrient (NO_3=10.98μM; PO_4=1.3μM; SiO_4= 11.4μM). Phytoplankton biomass was also sizably large at this depth (4 mg m^{-3}).

Previously, consortia of *Hemiaulus (hauckii)* and *R. intracellularies* has been reported abundantly (ca. 10^{10} cells m^{-2}) off the coast of South America in autumn, which led to an addition of about 24 mg N m^{-2} d^{-1} that resulted in an elevation of the primary production of the region Carpenter *et al.* (1999). Further, the ability of *R. intracellularies* to fix molecular nitrogen may simulate the development of the host diatoms in oligotrophic nutrient waters (Venrick, 1974). As reported in the Gulf of Aqaba similar association was recorded only during the stratified period and in the surface layers (Klinker *et al.*, 1978) where *H. membranaceus* cleve, *H hauckii* Grun and *H sinensis* Grev were found to harbor endosymbiont *Richelia intracellularis.* Apart from *Hemiaulus*, this cyanobacteria also known to live in other larger diatoms such as *Rhizosolenia*, wherein host is benefitted not only from the nitrogenous nutrient view point but also gets buoyancy due to the presence of gas vacuoles in the cytoplasm of endosymbiont -

Richelia, while host (diatom) provides protection inside the rigid frustules (Kimor *et al.*, 1992). Other regions where *Hemiaulus* demonstrated association with *R. intracellularis* include southern California and off Hawaii (Kimor *et al.*, 1978; Heinbokel, 1986).

6.3.1(i): *Amphisolenia bidentata with Synechococcus sp(possibly carcerarius (Plate 6I)*

The *Amphisolenia* is a distinct genus belong to dinoflagellate group and can be easily identified consisting of large fusiform cells (>1mm in length) with stick like figure and with inflated mid-body. This genus is predominantly distributed in tropical and subtropical waters (Sournia, 1970, Taylor, 1976).Work of Gul and Saifullah (2007), Subrahmayam and Sarma (1960), Noorudin (1967) and Taylor (1976) reported this genus from the Arabian Sea. This oceanic form sometimes found associated with upwelled waters in warm temperate to tropical waters (Tomas, 1997). Further, they are known to have the chloroplast of serial endo-symbiosis of other microalgae. In the present study, *Amphisolenia bidentata* was found associated with *Synechococcus* like cells (Plate 9) at 20m depth of stn 23 (and 24) forming ~ 3% of the total phytoplankton population (5460 cells l^{-1}). These stations located in the central Arabian Sea were influenced by the advected upwelled water characterized by low SST (26.3°C), high SSS (35.9psu), high NO_3-nutrient (~4µM) and low dissolved oxygen concentration (3.4 ml l^{-1}). Chlorophyll biomass at this depth was comparable to that in the surface layer (0.3 mg m^{-3}) possibly due to deeper mixed layer depth (~75m) of the station. Previous work of Hallegraeff and Jeffrey (1984) reports consortia of *Amphisolenia bidentata* and *A. thrinax* with *Synecococcus carcerarius* from the continental shelf waters of the north and North West Australia.

6.3.1 (j): *Dictyocha fibula with endosymbiont Synechococcus sp.(Plate 1J)*

Dictyocha cell are small (30-80µm in size including spines) solitary marine silicoflagellates found both in the coastal and oceanic areas, mostly in inshore waters. They abundantly occur in the Atlantic Ocean, Mediterranean, Baltic and the coast of Chile. Cells of this genus are often round/pyriformis with silica skeleton with four sides (in this case), and with four spines projecting from the corners, and from which fine pseudopodia usually project out. Generally, cell contains many plastids but with single emergent flagellum that helps in cell movement. In this

association, *Synechococcus* (like cells) were found inside the host cell either as single or in pairs (Plate 10). This association was found at sub surface depth (20m) of stn 16 (and at surface of stn 17) located closer to the Somalia coast. Waters of this depth were characterized by low temperature (25.7°C), low dissolved oxygen (3.3 ml L^{-1}), high salinity (33psu), high nitrate nutrient (4.5µM) and low chl *a* biomass (0.4 mg m^{-3}). The maximum cell abundance of this species (640 cells L^{-1}) contributed 8% (and 15% at stn 17) of the total phytoplankton community at stn 16. *Dictyocha* are also common in the coastal waters along the west coast of India particularly in winter (January) during which cell concentration goes as high as 2800 cell L^{-1} forming ca. 15% of the total phytoplankton community. In the Gulf of Aqaba, acantharians such as *Diplicoconus fasce*, *Amphilonche elongata* and *Acanthometra pellucida* are also known to have ectosymbiont (Michaels, 1991) contributing significantly to the primary productivity of the oligotrophic seas.

6.4: DISCUSSION

It is observed that most of the associations were found restricted to the upwelled (Somalia and Arabia coast) /advected waters extending up to the 67°E along 15°N latitude of the central Arabian Sea (Fig. 6.1 and Fig. 6.2A), region also received relatively more PAR (Fig. 6.2B).

Nitrogenase activity is known to be highly sensitive to and readily inactivated by molecular oxygen (Carnahan *et al.*, 1960; Postgate, 1982). To overcome this constrain some filamentous cells posses specialized cells termed heterocysts (Donze *et al.*, 1972), lack PS II and therefore produce no oxygen (Wolk, 1982), which obtain reduced carbon compounds derived from the neighboring vegetative cells to support N_2 fixation. On the other hand, non-heterocystous cells either undergo symbiosis or form aggregates (e.g. *Trichodesmium*) to obtain O_2 reduced microzones (see Paerl and Prufert 1987 and reference therein) to support N_2- fixation under aerobic conditions.

Dissolved iron in the ferrous (Fe (II)) oxidation state is required to support photosynthesis (e.g. in diatoms) and nitrogen fixation (e.g. in cyanobacteria). In general its

concentration is low in seawater (10^{-10}–10^{-9} moles Kg^{-1}; Geider, 1999). Recently, western AS is known to experience low in Fe concentration (Naqvi *et al.*, 2010) where most of the consortia were found. This raises the question (s) such as - what makes diatoms to host cyanobacterial endosymbiont in this region. Synechococcus is a group of unicellular cyanobacteria that thrives in all kinds of marine environments. Its size is around 1 mm, but this adaptation is highly successful in the pelagic environment because it enhances the efficiency of nutrient uptake and reduces the sinking rate (Raven, 1998). In addition, cyanobacteria are known to actively excrete strong iron-binding compounds (such as siderophores). However, it is not clear whether these siderophores help host cell to thrive in iron deplete condition. On the other hand, the work of Hutchins *et al.*, (1999) points out that the diatoms show a greater preference for iron from porphyrins than from siderophores.

Cyanobacterial associations with eukaryotes are ancient associations and are distributed widely in aquatic and terrestrial environment wherein symbionts provide a range of services (photosynthesis, nitrogen fixation, UV protection and defensive toxins) to host (Usher *et al.*, 2007). Among these, it was difficult for us to pin down actual motive that lead to these consortia. However, ability of symbiont to produce powerful defensive toxin that include antibiotics and antifeedants (Borowitzka and Hinde, 1999) may protect host from small heterotrophic nanoflagellate, and larger predators such as copepods that are abundantly found in the western Arabian Sea than to the east. On the other hand, symbionts (such as cyanobacteria, which are generally small in size) get protection inside the frustules particularly from micro-grazers such as microzooplankton (and smaller mesozooplankton). Microzooplankton that are abundantly found in the western AS are known to consume significant portion of phytoplankton standing stock (Landry *et al.*, 1998). Apart, the cyanobionts are also known to provide UV protection to their host partners, which will be particularly important during summer period. Cyanobacteria produce two types of sunscreen compounds, mycosporine-like amino acids (Shick and Dunlap, 2002) and scytonemin (Proteau *et al.*, 1993), both of which contain N. Thus, host is benefited both from the incoming solar insolation and from the N-source.

6.5: CONCLUSIONS

Present study reports instance of symbiotic association in the phytoplankton of the Arabian Sea (coastal and open waters) observed during Roger Revelle cruise of 2007 and at the CaTS time series location for the period of 2005-06. During the present study association were found higher in the open waters than in the coastal region. Phytoplankton with associations observed similar to present study is being overlooked particularly endosymbiotic as samples are rarely examined onboard research cruises and sample preservation with Lugol's Solution may mask fluoroscence. Secondly, fluroscence microscopy is typically used in for picoplankton research and larger diatoms and dinoflagellates are rarely examined as pointed out by Villareal, 1992. Picoplankton samples in these type associations represent an important and previously unrecognized source of new N to support primary production in nutrient poor tropic waters. Furthermore, this bloom demonstrates that heterocystous cyanobacteria can also make quantitatively important contributions of N in oceanic water column environment. In the study area such associations possibly occurring regularly particularly during spring and fall intermonsoon when system undergo oligotrophy. Further, both diatoms and dinoflagellates that are known to have associations elsewhere do occur in our waters both coastal and open ocean thereby adding new nitrogen to the system and balancing the apparent deficiency in N inputs as observed in studies of Sambrotto *et al.*, 1993; Michaels *et al.*, 1996, Gruber and Sarmiento, 1997). The quantitative importance of these associations in oceanic Nitrogen cycling has not been assessed. Interestingly, none of the association was found in bloom form (Carpenter *et al.*, 1999). Considering the global warming associated stratification in near future, association with diazotrophic endosymbionts may constitute an important source of nitrogen to support primary production. Nonetheless, such associations need to be closely monitored at time series locations like CaTS to document their occurrences with respect to biogeochemistry of the region.

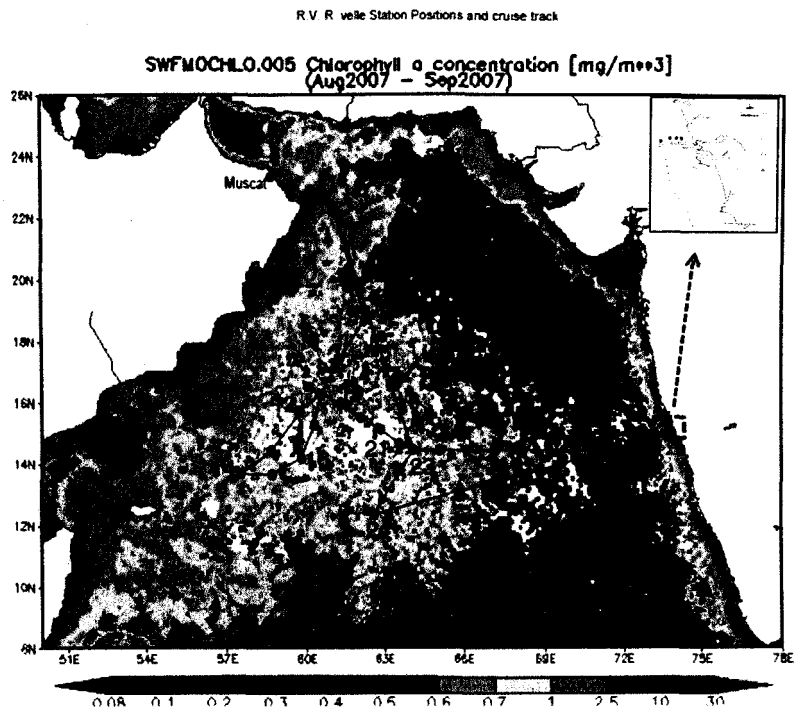


Fig. 6.1: Map showing SeaWiFS Chl a image for August-September 2007 (source:http://gdata1.sci.gsfc.nasa.gov/daac-bin/G3/gui.cgi?instance_id=ocean_month) with superimposed station locations along the transect. Rectangular box indicate station locations along the Candolim Time Series (CATs) transect.

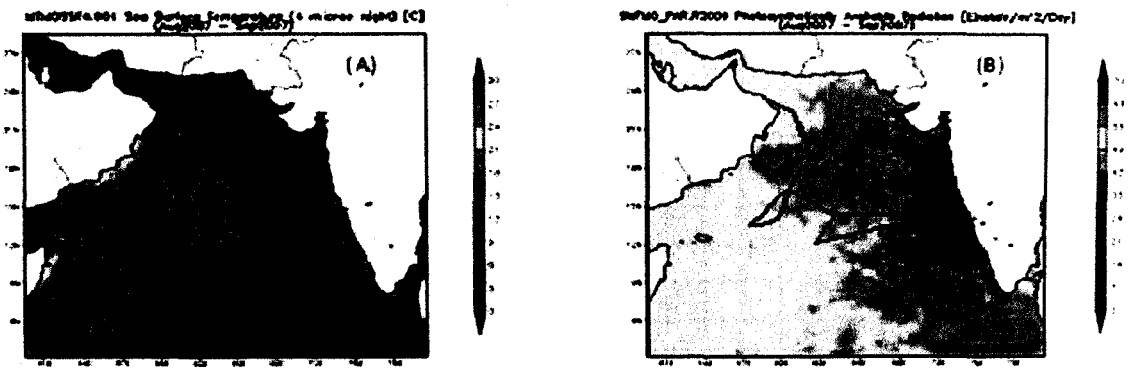


Fig. 6.2: Map showing SeaWiFS Chl a image for August-September 2007 (source:http://gdata1.sci.gsfc.nasa.gov/daac-bin/G3/gui.cgi?instance_id=ocean_month) with superimposed ship tract. Satellite - derived (A) SST (oC) (B) Photo synthetically available radiations (C) Particulate organic carbon and (D) Particulate inorganic carbon along the RR cruise transect.

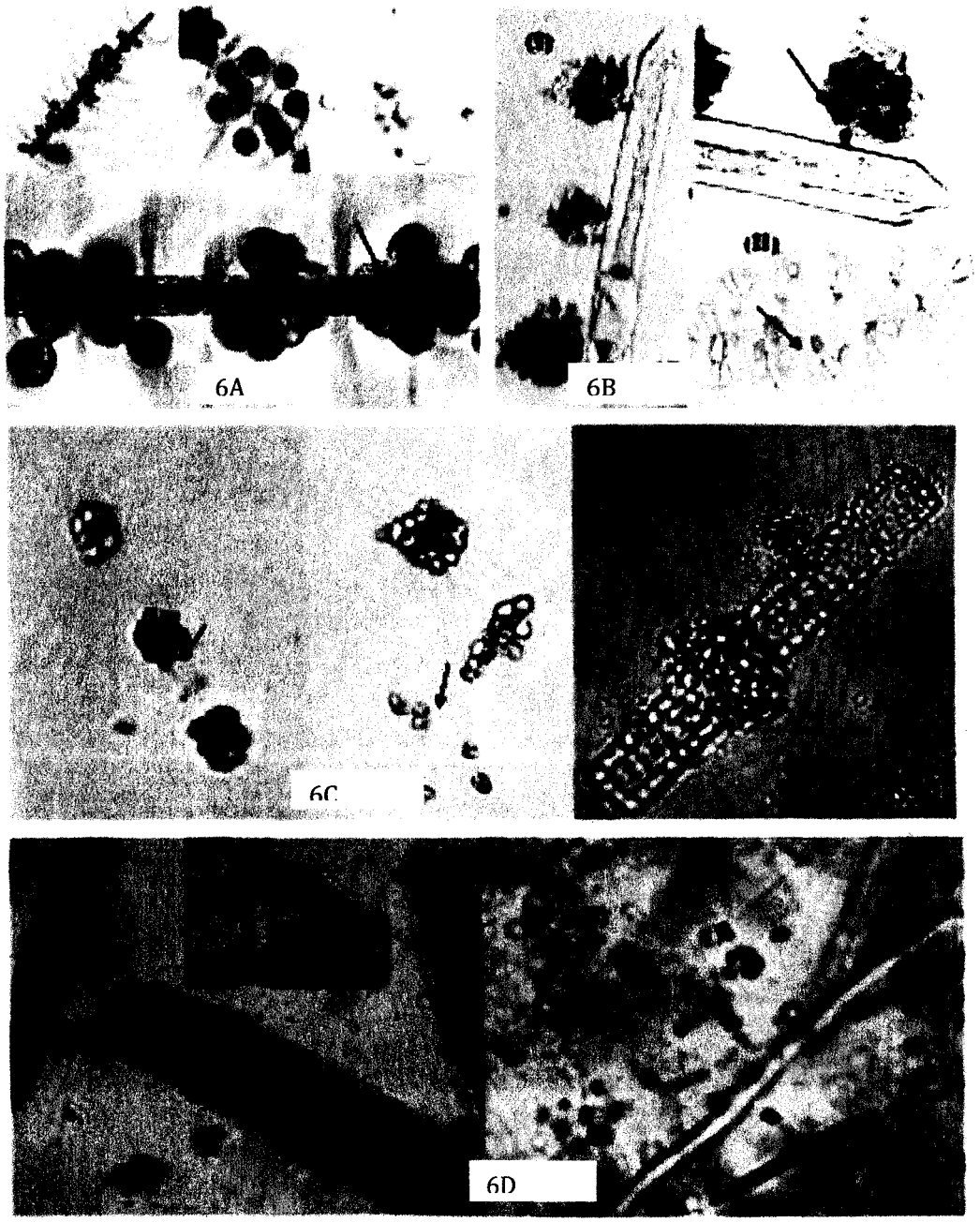


Plate- 6(A-D): (A) diatom *Chaetoceros coarctatus* associated with epiphytic *Vorticella oceanica*; **(B) I-** diatom *Rhizosolenia alata* with epiphytic *Vorticella* sp, **(II) -** a colony of *Vorticella* sp.; **(C)** diatom *Leptocylindrus mediterraneus* with epiphytic flagellate *Solenicola setigera* ;**(D)** diatom *Coscinodiscus gigas* association with epiphytic protozoan like cells.

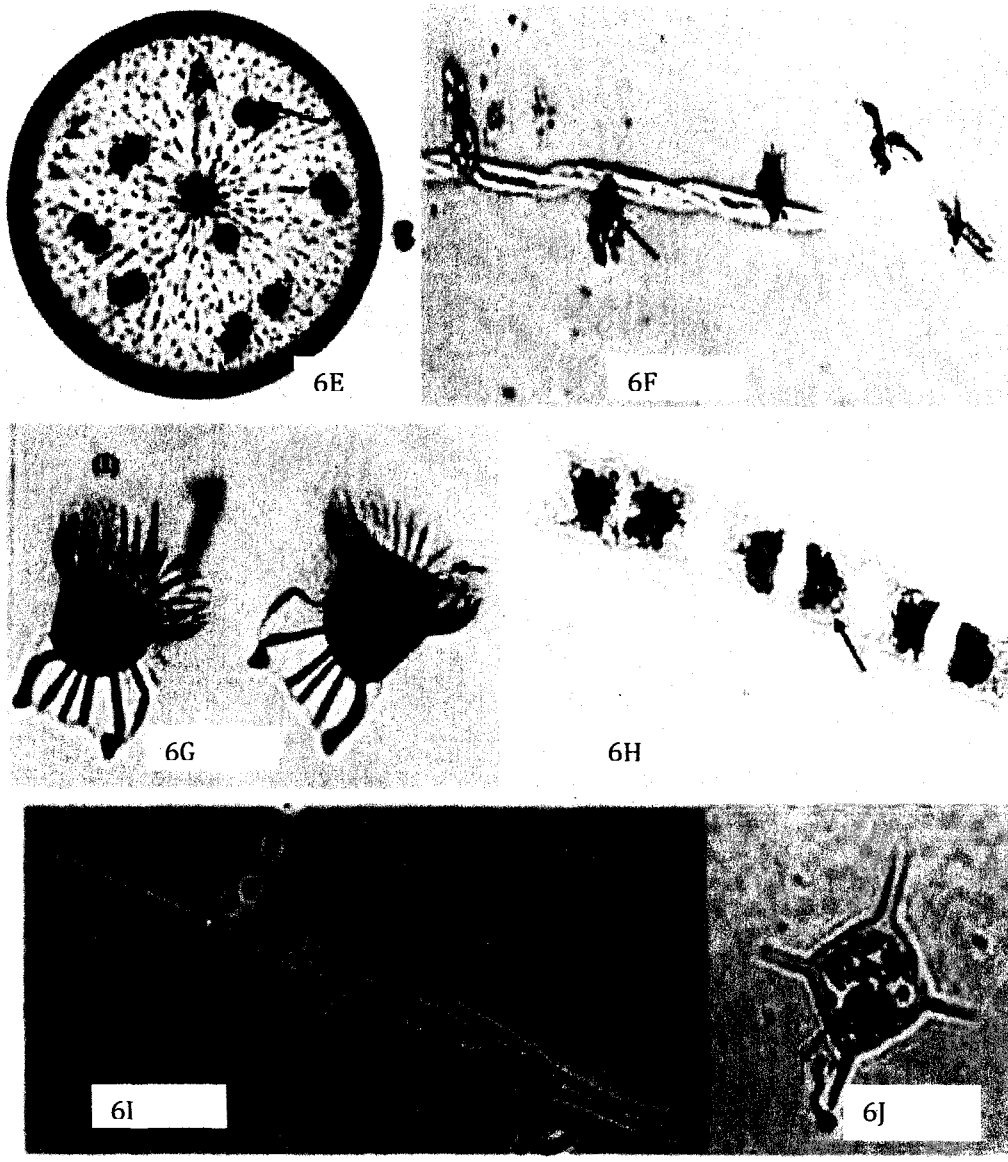


Plate- 6(E-J): (E) I- *Trichodesmium erythraeum* association with epiphytic unidentified globular cells, (II) -bacterial community associated with the senescent phase of the bloom; **(F)** diatom *Pseudonitzchia seriata* association with epiphytic unidentified forms; **(G)** the dinoflagellate *Ornithocercus quadrates* with endocytic association of *Synechococcus* sp.; **(H)** diatom *Hemiaulus membranaceus* endosymbiotic association with cyanobacteria *Richelia intracellularies* ; **(I)** dinoflagellate *Amphisolenia bidentata* with endosymbiotic cyanobacteria; **(J)** silicoflagellate, *Dictyocha fibula bidentata* with endosymbiotic cyanobacteria

CHAPTER 7

SECTION-7A

EFFECT OF NUTRIENT ENRICHMENT ON GROWTH OF THE PHYTOPLANKTON IN THE ZUARI ESTUARY, INDIA

7A.1: INTRODUCTION

Phytoplankton are responsible for nearly half of global primary production (Falkowski *et al.*, 1998). A substantial proportion of the coasts include highly productive estuaries (Boto *et al.*, 1984; Wafer *et al.*, 1997). These estuaries, besides supporting a wide variety of animals and plants, act as an important linkage and buffer zone between the ocean and land. They are also sites of high rates of production of organic matter, which not only sustain a secondary food chain internally, but also influence biological productivity of adjacent coastal water in turn sustaining fisheries (Robertson and Alongi, 1992; Wafer *et al.*, 1997). The extent to which estuaries exchange dissolved and particulate nutrients with adjacent marine ecosystems depend upon several factors, including geomorphology, tidal regime, climate and fresh water inputs. Light and nutrients are the primary factors regulating phytoplankton growth followed by the temperature and salinity (Devassy and Goes, 1988). The major (macro) nutrients essential for plant growth are nitrogen, phosphorous and silicon (Ryther and Dunstan, 1971). Phytoplankton preference for reduced N compounds, primarily as ammonium and urea, is an almost universal phenomenon in marine systems, including estuaries (e.g. McCarthy *et al.*, 1977; Glibert *et al.*, 1982; Probyn, 1985).

In many coastal waters, increasing eutrophication, due to human activities has greatly perturbed the phytoplankton community. Fixation of dinitrogen is yet another phenomenon of ecological significance known to naturally fertilize tropical waters (Devassy *et al.*, 1978). The

consequences of eutrophication can only be minimized by identifying the specific nutrient that is limiting to algal growth and primary production. In case of fresh water systems, it is phosphorus (Schindler, 1977), whist in marine system it is generally nitrogen (Tyrell and Law, 1997). However, a seasonal shift from phosphorus to nitrogen limitation is observed in coastal transition areas, such as estuaries (Malone *et al.,* 1996) while limitation in bio available silica is also been reported from subtropical estuary of Taiwan (Wu and Chou, 2003).

Western Indian shelf experiences SW monsoon induced coastal upwelling during June to September (Shetye *et al.*, 1990) which results in nutrification of the shelf water. This leads to enhanced primary productivity. However due to a unique interplay of hydrographical and biogeochemical condition, shelf water experience hypoxia from June to July, suboxia from August to September and anoxia (sulfidic condition) during October (Naqvi *et al.*, 2006). This nutrient rich low oxygenated upwelled water enters the mouth region of Mandovi-Zuari estuarine system (Sankaranarayanan and Jayaraman, 1972) which is expected to affect plankton ecology of the estuarine system. Phytoplankton in the shelf water experience N-limited condition during non-monsoon period (November to May), but N enrichment sets in from June through upwelling. Due to prevailing rough weather during this period, no expedition has been possible to the shelf in order to carry out any study to assess the response of algal biomass to such natural N enrichment. Thus it is unknown how quick is the algal response to the nutrient enrichment and how it changes the growth and taxonomy of the algal biomass. Also it is unknown, how adaptive are the plankton to the low oxygen condition over the shelf during this period.

In the present study, we simulated the effect of upwelling by artificially enriching the estuarine water with inorganic nutrients, in order to understand the dynamics of algal nutrient uptake in response to nutrient enrichment and its growth and to determine nutrient limitation, if any. Zuari estuarine system is a well-mixed coastal-plain monsoonal estuary situated between latitudes 15° 25' to 15° 31' N and longitudes 73° 45' to 73° 59' E in Goa, along the west coast of India. The microcosm experiment was carried out in the near mouth river of Zuari as depicted in the Fig. 7A.1.

7 A.2: METHOD

7 A.2.1: SAMPLING AND ANALYSIS

Before the incubation experiment, ambient water samples were collected and analyzed for a range of parameters such as pH, temperature, salinity, dissolved oxygen, nutrients, microzooplankton counts, phytoplankton pigments, composition and abundance. All samples collected were processed following standard protocols described in detail in chapter 3. But sample for DO in this experiment was done by using spectrophotometer and pigment compositon using HPLC technique the details are given below.

7A.2.1. 1: PIGMENT COMPOSITION (HPLC BASED)

For pigment analysis, water samples (200-300 mL) were filtered through GF/F filters (0.7 μm, 25 mm diameter) and then stored at -85°C until analysis. Extraction of pigments was done in 3 mL of 95% acetone for 5 min in an ultrasonic bath that was filled with ice-water. The extracts were then stored overnight at -20°C for HPLC (high performance liquid chromatography) analysis. Entire extraction procedure was carried out in dim light and at low temperature to minimize degradation of pigments. The HPLC analysis was carried out following the method of Van Heukelem (2002) as detailed in Roy *et al.*, (2006).

7A.2.1. 2: DISSOLVED OXYGEN

Samples for oxygen particularly for experimental purpose were collected in gas tight Hamilton glass syringes and were fixed immediately by adding Winkler A and Winkler B solution. The precipitate i.e. Mn $(OH)_3$ was subsequently dissolved by acidification and the absorbance of developed colour was measured at 456nm (Pai *et al.*, 1993) by a Shimadzu UV-visible spectrophotometer using 1 cm cuvette. A reagent blank was prepared by adding the acid, Winkler's B and Winnkler's A solution (in reverse order) to a volume of distilled water equal to that of the samples and the resultant absorbance at 456 nm was subtracted from the absorbance of sample. Precision of the oxygen measurement by this method is about 0.1%.This technique is particularly useful and more precise for dissolved oxygen measurements at low concentration.

7A.2.3: EXPERIMENTAL SET-UP AND *IN-SITU* INCUBATION

Two nutrient enrichment experiments were conducted *in-situ* during February and March 2006. Nalgene bottles (25.5L capacity) were modified (Fig. 7A.2) for this purpose by drilling two holes through the cap of each bottle. Two nylon tubes were inserted; one reaching the bottom of the bottle to draw the sample and the other was kept just below the cap to replace the volume of the water removed with helium. The outer ends of each tube were fitted with a three way valve and the entire system was ensured to be airtight (Fig. 7A.2). First experiment was done in February. Water samples from 1m depth were drawn using a Niskin sampler and screened slowly and carefully through a 200 μm nylon mesh to exclude macrograzers, without creating much turbulence to avoid damage to delicate organisms such as ciliates. The first bottle (Bottle-A) was enriched with NO_3^-, PO_4^{-3}, SiO_4^{-4} and NH_4^+ , the second bottle (Bottle-B) was spiked with NO_3^-, PO_4^{-3}, and SiO_4^{-4} and the third bottle (Bottle-C) was used as a control without any addition of nutrients. The ambient concentrations of nutrients (μM) before incubation were NO_3^- = 0.51 μM, NO_2^- = 0.09 μM, PO_4^{-3}= 0.57 μM, SiO_4^{-4} = 10.92 μM and NH_4^+ = 2.28 μM. The concentrations were enhanced to 11.7 μM NO_3^- , 0.93 μM PO_4^{-3}, 18.9 μM SiO_4^{-4} and 4.5 μM NH_4^+. The bottles were deployed at 1m depth by hanging from a moored floating raft. The first sampling was done after 16 h of incubation and subsequently after every 24 h. Samples from each bottle were drawn almost at the same time using plastic BD syringes. The volume of the water drawn was replaced simultaneously with helium from air tight gas bags. The incubation lasted for ~11 days.

The second experiment (experiment-2) was conducted in March, wherein bottle-A was enriched with NO_3^-, PO_4^{-3}, and SiO_4^{-4} and second bottle, bottle-B, was enriched with nutrients similar to bottle-A but deoxygenated by purging helium gas. This bottle was maintained at hypoxic level (<2 mL O_2 L^{-1}). While bottle-C served as a control. The physico-chemical characteristics of the estuary were quite similar to those in February and the ambient nutrients concentration of NO_3^-, NO_2^- , NH_4^+, PO_4^{-3}, and SiO_4^{-4} were 0.35, 0.12, 0.46, 0.67 and 8.41 μM respectively. Relative to experiment-1, samples were collected at much closer time intervals i.e. every 4 hourly during the first 32 h; and subsequently at 24 hourly interval for a period of 5 days.

7A.3: RESULTS

7A.3.1: EXPERIMENT -1 (2-13 FEBRUARY 2006)

7A.3.1. 1: NUTRIENT UPTAKE

This experiment was initiated during high tide (2.07m, 13:40 h), and the bottles were incubated *in situ* at 16:00 h. The first sampling (T1) was done after 16 h of incubation. Due to the delayed incubation on the first day, only 3 hours sunlight was available for photosynthesis, hence there was no significant decrease in nutrient concentrations. Bottles A and B showed a drastic drop of NO_3^-, PO_4^{-3} and SiO_4^{-4} levels between 16 and 40 h of incubation. The decreases in nutrient concentrations coincided with a sharp increase in chlorophyll *a* concentration. In the control bottle (bottle-C) the chlorophyll concentration decreased with time (Fig. 7A.3). The phytoplankton biomass which showed an increase by 23 µg chl *a* L^{-1} resulted in utilization of 10 µM nitrate, 17 µM silicate and 2.2 µM ammonium in bottle-A. Similarly, in bottle-B chlorophyll increased to 22.5 µg chl *a* L^{-1} with concomitant utilization of 8 µM nitrate, 0.6 µM phosphate, 15 µM silicate and 0.8 µM ammonium after 16 h of incubation.

After 40 h of incubation 21 µg chl *a* L^{-1} resulted in the utilization of 12 µM, 0.6 µM, 19 µM and 2.4 µM of NO_3^-, PO_4^{-3}, SiO_4^{-4}, and NH_4^+, respectively, from the initial concentrations in bottle- A. While, in bottle-B an increase by 20 µg chl *a* L^{-1} resulted in utilization of 11 µM, 0.34 µM, 19 µM and 1 µM of NO_3^-, PO_4^{-3}, SiO_4^{-4} and NH_4^+ respectively.

Thus, after 16 h of incubation (up to 64 h) concentration of nutrients in bottle-A decreased by 84-98% for NO_3^-, 58-66% for PO_4^{-3}, 88-100% for SiO_4^{-4} and 50-54% for NH_4^+ and in bottle- B, NO_3^- decreased by 65-94% , PO_4^{3-} by 34-61%, SiO_4^{4-} by 77-100% and NH_4^+ by 30-45% of the original values while chlorophyll *a* increased by a factor of three in both the bottles. It clearly indicates that NO_3^- and SiO_4^{-4} were nearly exhausted after 40 h of incubation (Fig. 7A.3). For every 1 µM decrease in NO_3^- resulted in 2.3-2.9 (avg. 2.6) µg L^{-1} chl *a* gain in phytoplankton biomass. This gain was particularly seen between 16 and 40 h of incubation period. Though NH_4^+ was also taken up by phytoplankton along with NO_3^- (and NO_2^-), the uptake rate was comparatively much lower. However, after 3.5 days when NO_3^- had been

depleted, NH_4^+ was still available in the medium that perhaps resulted in secondary chlorophyll peak in bottle-A (17 μg L^{-1}) at 136 h and in bottle-B (8 μg L^{-1}) at 88 h coinciding with the decline in NH_4^+ concentration.

The enclosed water remained well oxygenated (>4 mL O_2 L^{-1}) throughout the experiment. Hence, the decrease in NO_3^- should be entirely due to the uptake by phytoplankton rather than by anaerobic respiratory process such as denitrification. NO_3^- was significantly preferred over NH_4^+ by phytoplankton. The difference in NO_3^- uptake pattern in bottle A and B was found to be insignificant (ANOVA, p= 0.9) indicating that the uptake pattern of nutrients and biomass growth were similar in both the bottles. The variation between the data sets for all the parameters between two bottles were insignificant (p>0.05) indicating bottle-A and bottle-B behaved almost as duplicates. Net growth rates (μ) from changes in the chlorophyll biomass (Pedersen and Borum, 1996) were calculated to be 1.24 and 1.23 μg chl L^{-1} d^{-1} in bottle A and B respectively. This closeness of μ in both bottles indicates that presence of NH_4^+ did not cause any significant change in algal biomass as long as NO_3^- was available, and at the same time NH_4^+ did not suppress uptake of NO_3^- in bottle-A. While bottle-C showed -0.67 μg chl L^{-1} d^{-1} due to high grazing pressure exerted by the microzooplankton; tintinnids in particular and lack of nutrients to support further build up of phytoplankton biomass (Fig. 7.8).

7A.3.1. 2: PHYTOPLANKTON COMPOSITION

It was observed that chlorophyll *a* in both the treated bottles responded in similar pattern. Likewise, the phytoplankton abundance also showed similar trend except at 40 h that accounted to sudden increase in abundance in bottle-B as compared to bottle-A (Fig. 7A.4a). This high value in bottle-B was due to the outburst of *Thalassionema nitzschioides*, *Thalassiosira subtilis*, *Asterionella japonica* and *Chaetoceros curvisetus* (Fig. 7A.4b).

The phytoplankton assemblage was composed of 42 species (diatoms-35; dinoflagellates-6, and silicoflagellate-1) in the experimental bottles. Cell density varied from $1.4x10^5$ to $3.0x\ 10^6$ cells L^{-1} in bottle-A and from 1.1×10^5 to 8.7×10^6 cells L^{-1} in bottle-B. The control bottle

showed a range from 1.2 x10^5 to 4.6x 10^5 cells L^{-1} in 40 h. The diatoms accounted for 99% of the total phytoplankton community. The dominant species were *Thalassionema nitzschioides*, *Thalassiosira subtilis*, *Asterionella japonica*, *Chaetoceros curvisetus*, *Melosira sp.*, *Skeletonema costatum*, *Chaetoceros lorenzianus*, *Guinardia striata*, *Guinardia delicatula*, *Leptocylindrus danicus*, *Pseudo-nitzschia seriata*, *Dactylisolen sp* and *Nitzschia closterium*. Among these, *Thalassionema nitzschoides*, *Asteronella japonica*, *Pseudo-nitzchia seriata*, and *Nitzschia closterium* are chain-forming pennates while others are centric diatoms. However, dinoflagellates like *Ceratium sp*, *Gymnodinium sp*, *Prorocentrum spp.*, and *Protoperidinium spp.* were negligible to the total community. *Dictyocha fibula* was the only representative belonging to silicoflagellates.

7A.3.2: EXPERIMENT -2 (25-30 MARCH 2006)

7A.3.2. 1: NUTRIENT UPTAKE

In the first experiment the bottles were exposed to sunlight only for ~ 3 h after their deployment. The reason for low uptake of nutrients during the first 16 h i.e., whether it was due to non-occurrence of photosynthesis at night or a time lag in phytoplankton response could not be ascertained. Therefore, this experiment was repeated to resolve this issue. In addition, an experimental bottle (Bottle-B) was maintained at low oxygen concentration (O_2<2 mlL^{-1}) but enriched with nutrients. This was to determine the response of phytoplankton cells when exposed to deoxygenated waters, as happens naturally during the southwest monsoon when the study area experiences incursion of upwelled waters (Sankaranarayanan and Jayaraman, 1972). In this experiment, the bottles were exposed to sunlight for whole day light period (11 h). All the bottles were deployed early in the morning and sampling was done at much shorter time intervals (every 4 hours) within the first 32 h to closely monitor nutrient uptake pattern and thereafter every 24 h intervals.

During this experiment, all bottles were incubated well before sunrise after enriching with nutrients. Sharp decreases in nutrients (NO_3^- by 9 µM; PO_4^{-3} by 0.3 µM and SiO_4^{-4} by 12 µM) with a concomitant increase of 19 µg L^{-1} chl *a* in the bottle-A at ~ 32 h of incubation was observed (growth rate = 1.4 µg chl *a* L^{-1} d^{-1}). As expected, changes in these parameters were

negligible in the control, bottle-C (Fig. 7A.5), and the difference between the initial and final nutrient and chlorophyll concentrations were still found to be statistically insignificant (ANOVA, $p= 0.9$).

Bottle-B was suddenly subjected to hypoxic conditions, attained within 1 hr by purging with helium gas. The phytoplankton abundance were found to be low, but overall their biomass was relatively higher than control bottle and responded in similar manner as in bottle-A. Likewise, dissolved oxygen in deoxygenated bottle was low initially (<2 mL O_2 L^{-1}) but, after 4-8 h of incubation oxygen level increased to ~3 mL O_2 L^{-1} and remained consistent throughout the experiment except at nights. The buildup of oxygen in the bottle coincided with the increase in chlorophyll, although cell counts were low. A close coupling between chlorophyll *a* and dissolved oxygen due to photosynthetic activity is shown in (Fig. 7A.6). The NH_4^+ concentration in bottle-B dropped by 50% while in other bottles it showed only 5% decline, while NO_3^- and SiO_4^{-4} decline was negligible with only 8 and 3% respectively to their initial concentration in bottle- B. On the whole, substantial nutrient decline and chlorophyll buildup (i.e. 5 to 30 µg chl *a* L^{-1}) with rapid uptake occurred only after 20 h of incubation earlier by 4 h in deoxygenated bottle as compared to other bottles. The growth rate was found to be comparatively higher with 1.6 (µg chl *a* L^{-1} d^{-1}) in bottle- B than in control bottle (0.6).

7A.3.2.2: PIGMENT COMPOSITION

Apart from chlorophyll *a,* HPLC analysis revealed presence of other marker pigments such as chl c_2, chl *b,* fucoxanthin, zeaxanthin peridinin, diadinoxanthin, diatoxanthin, alloxanthin, and α+β carotene. Combination of marker pigments suggests that chl *a*, chl c_2, fucoxanthin (diatom) and zeaxanthin (cyanobacteria) were the dominant groups that contributed to the total phytoplankton biomass in this enrichment experiment. The percentage distribution of major pigments that attained maxima at 32 h after enrichment is shown in Table 7.I. The other minor pigments *viz.* diadinoxanthin, alloxanthin, diatoxanthin, alpha carotene and peridinin collectively accounted for 2-3%. However, peridinin which is the marker pigment for autotrophic dinoflagellates were negligible in all bottles. In general, most of the other pigment compositions in all bottles remained similar but varied in concentrations.

7A.3.2.3: PHYTOPLANKTON COMPOSITION

The phytoplankton composition was almost similar to experiment-1 and composed of 45 species (diatoms-28; dinoflagellates-7, silicoflagellate-1 and blue green algae-1). Cell density in bottle- A varied from 6.6 $x10^4$ to 7.6 x 10^5 cells L^{-1}. The control bottle showed a range from 3.5 $x10^4$ to 4.9x 10^5 and in bottle-B counts ranged from 2.7x 10^4 to 5.8x 10^5 cells L^{-1} in 32 h. In general, diatom accounted for 98% of the total algae community. This high values was again due to the dominance of fast growing chain forming diatoms *viz. Skeletonema costatum, Thalassionema nitzschioides, Chaetoceros curvisetus, Chaetoceros lorenzianus, Leptocylindrus danicus* and *Guinardia striata.* Further, in all bottles including bottle-B these species showed gradual increase after 4 h which coincided to the rise in chlorophyll *a* and oxygen production. Conversely, some forms *viz. Thalassiosira subtilus, Ditylum brightwellii, Melosira* sp and *Rhizosolenia crassispina* were low in abundance in bottle-B as compared to the control bottle. (Fig. 7A.7a) while *Skeletonema costatum, Chaetoceros curvisetus* and *Thalassionema nitzschioides* were the only forms that remained invariably high in both the treated bottles-A and B (Fig. 7A. 7b).

In general, the phytoplankton composition did not vary much among all the bottles. However, numerically cells were higher by 70% in bottles-A and B compared to the control bottle. Similar to the first experiment, maxima in phytoplankton density, was observed at 32 h of incubation, clearly coincided with high chl *a*, which decreased gradually with time and was comparable to control bottle after 182 h. Diatoms always remained the dominant group comprising >96% of the algal community. Dinoflagellates, silicoflagellates and diazotroph *Trichodesmium erythraeum* collectively formed < 5% of the total phytoplankton community.

7A.4: DISCUSSION

Results of incubation experiments provide valuable insights into the response of natural phytoplankton assemblages to nutrient enrichment. Previous results from enclosure experiments have been useful to describe processes operating in natural conditions (e.g. Pitcher *et al.*, 1993).

Such experimental approach has revealed how nutrient limitation may affect algal growth rate and net biomass accumulation. Nutrients are largely assimilated by phytoplankton during the day for photosynthesis. From the above two experiments, it is apparent that the response of estuarine phytoplankton to nutrient enrichment is almost immediate. An increase in 19-26 (avg. 23) μg chl *a* L^{-1} resulted in loss of 8-10 (avg.9) μM NO_3^-, 0.3-0.6 (avg.0.45) μM PO_4^{-3} and 9-17 (avg.13) μM SiO_4^{-4}. The observed rapid phytoplankton uptake within 24 h of nutrient enrichment in the study region and may be true for other tropical estuaries. There does, however, appear a period of few hours when the uptake is relatively slow, as observed particularly during the experiment-2 conducted in March. The lower initial uptake rate may be due to physiological adaptation of phytoplankton to eutrophication.

Studies with unialgal laboratory cultures and artificially enriched coastal seawater have shown that marine phytoplankton prefer NH_4^+ over NO_3^- as a nitrogen source (McCarthy and Eppley, 1972). Several other authors (Probyn and Painting, 1985; Dortch and Postel, 1989) have also reported the inhibitory effect of NH_4^+ on NO_3^- which severely reduces the rate of NO_3^- uptake. Furthermore, Paasche and Kristiansen (1982) and Dortch and Conway (1984) found a threshold ammonium concentration of 1 μM, above which NO_3^- uptake is largely inhibited despite high concentration of ambient NO_3^-. Similar conclusion was drawn based on the theoretical consideration of the relative energy requirement for the utilization of NO_3^- and NH_4^+ (Losado and Guerrero, 1979; Syrett, 1981). Some reports have shown simultaneous uptake of NO_3^- and NH_4^+ (Harrison *et al.,* 1983; Price *et al* 1985). But, unlike others, a preference for NO_3^- over NH_4^+ was also observed (Wafer *et al.*, 1983). In nitrogen replete cultures, studies have also shown enhanced metabolism of NO_3^- with increase in irradiance (Lomas and Glibert, 2000). Other studies have found that, the half saturation constant for nitrate uptake was related to temperature (Eppley *et al.,* 1969) where, higher temperatures enhance NO_3^- utilization (Dham *et al.*, 2005). In our study, in case of experiment-1, NH_4^+ concentration was >2 μM, but no inhibition was observed in the bottles A and B. Instead, we found that NO_3^- was taken up before NH_4^+ and the uptake pattern of nutrients and biomass growth appeared to be similar in bottle-A and bottle-B. No significant difference was found between these two data sets ($p>0.05$). These results suggest that the phytoplankton community in the Zuari estuary preferred NO_3^- over NH_4^+.

Large sized phytoplankton cells preferentially assimilating NO_3^- over NH_4^+ (Malone, 1980); Kokkinakis and Wheeler, 1987 and 1988). Studies on nitrogen uptake by size-fractionated plankton showed that the NO_3^- was utilized by net-plankton and NH_4^+ by nanoplankton (0.8-20 µm size) (Wafar *et al.,* 2004; Dham *et al.,* 2005). Similarly, in this study most of the dominant taxa were the fast growing diatoms (>10 µm size) that preferred NO_3^- while others smaller may have preferred NH_4^+. Several authors (Glibert *et al.,* 1982; Smith and Nelson, 1990 and Owens *et al.,*1990) have used relative preference index, RPI to study the preferential N uptake. A consistent value of RPI (>1) for NO_3^- in both experiments indicates that NO_3^- is the preferred N substrate for phytoplankton in the estuary irrespective of higher ambient NH_4^+ concentration. (Table 7.II).

Diatoms have evolved a multitude of morphologies, which serve as protection against grazing or sinking (Smayda, 1970). The impact of cell shape and chain forms can be used to predict uptake of nutrients under turbulent environments (Pahlow *et al.*, 1997). Silicate an essential nutrient for diatoms was in surplus to support their build up. But high silicate and low nitrate as in this estuary might limit the phytoplankton growth. Therefore, in this experiment, silicon and nitrogen were added in equal proportions as most diatoms incorporate in their molar ratio of about 1:1 (Brzezinski, 1985). Small-scale nitrogen enrichment in cultures has shown a rapid increase in diatom, namely *Skeletonema costatum* (Balode et al, 1998). Likewise, in these experiments, fast growing dominant taxa, *viz. Thalassionema nitzschioides, Thalassiosira subtilis, Asterionella japonica, Chaetoceros curvisetus, Chaetoceros lorenzianus, Guinardia striata, Guinardia delicatula, Skeletonema costatum* and *Leptocylindrus danicus* showed momentous increase and accounted for >96% of phytoplankton preferentially used nitrate. The HPLC analysis revealed the dominance chl *a* (total algal biomass including cyanobacteria) and of larger cells especially diatoms (fucoxanthin). However, it also indicates the presence of pigments such as zeaxanthin, alloxanthin and chlorophyll *b*. These are the marker pigments of cyanobacteria, cryptophytes and green algae respectively, which were underestimated by the microscopic analysis presumably because of smaller cell size. Diatoms always remained the dominant group comprising >96% of the total algal community indicating the efficiency of this community in nutrient utilization. However, in experiment-1 the subsequent decrease in the

chlorophyll after 32 h possibly occurs due to grazing pressure exerted by the micro-grazers such as ciliates in particular (Fig. 8).

Phytoplankton species *viz.*, *Melosira* sp., *Rhizosolenia crassispina* and *Ditylum brightwellii,* were a few forms found to be initially low in abundance when oxygen level was < 2 mL O_2 L^{-1} (Fig. 7A.7a), but their biomass was higher and they showed an increase after 24 h indicating that they have potential to bloom given the right conditions. This suggests that, larger forms were under stress but the pico and nano fractions responded efficiently under low oxygen conditions utilizing NH_4^+ concentration which dropped to 50% of its initial value after 4 h in bottle-B. Thereafter the oxygen level was restored through rapid photosynthesis. This may be happening to the phytoplankton community in the water column during post southwest monsoon supporting the smaller forms *viz.* pico community through regenerated nitrogenous input.

Interestingly three species namely *Skeletonema costatum, Chaetoceros curvisetus* and *Thalassionema nitzschioides* remained invariably high in bottle-B (Fig. 7A.7b), these species picked up after 4 h of incubation unlike other diatoms signifying that these species may be thriving in environments such as along the western continental shelf of India, which experiences oxygen deficiency (hypoxia, suboxia followed by anoxia) during southwest monsoon period (Naqvi *et al.*, 2000). In support of this view, there is data reported from this study area showing few phytoplankton like *Ceratium* spp., *Gymnodinium* sp., *Gyrodinium* sp. (dinoflagellates), *Pseudo-nitzschia* sp. *Navicula* spp., *Thalassiothrix* sp., *Thalassionema* spp. (pennate) and *Coscinodiscus* sp, *Chaetoceros spp., Skeletomema* sp., and *Pleurosigma* sp. (centric), prevailing during this time of the year (unpublished data).

The presence of considerable amount of NH_4^+ (>2 μM) did not inhibit the rate of NO_3^- uptake by phytoplankton. Though in the experiments NH_4^+ did not seem to contribute directly to the growth of biomass, as NO_3^- was the preferred species, still its conversion to NO_3^- via nitrification, can indirectly meet the N requirement of phytoplankton. Further, NH_4^+ can also be made available again through re-mineralization, as seen after 5 days of incubation in experiment-1 which boosted the secondary chlorophyll peak. It also leads to speculation that NH_4^+ is taken up significantly when ambient NO_3^- concentration is low. This speculation arises from the fact

that although NO_3^- remains low (<1 μM) as compared to PO_4^{-3} (>0.5 μM) or SiO_4^{-4} (>8 μM) in the non monsoon seasons, maximum primary production occurs during the premonsoon (March-April) and post monsoon (Oct-Nov) periods (Krishnakumari *et al.,* 2002; Ram *et al.*, 2003) which is supported through regenerated nutrients such as NH_4^+.

However, the DIN in the Zuari estuary has been observed to remain low unless there are some episodic inputs of nutrients. Molar ratios of nutrients in the water at time of experiment -1, (DIN/DIP= 5.05; and DIN/Si= 0.26) and in experiment -2 (DIN/DIP= 1.39; and DIN/Si= 0.11) was much lower than the Redfield values suggesting N limitation in this region during the pre-monsoon season. The nutrient uptake of nitrate, phosphate and silicate by the phytoplankton community was taken up close to the Redfield ratio in February and March (Table 7. III). However higher DIN: DIP (>16) in March indicates that nitrogen is assimilated at slightly faster rate (Table 7. II) possibly due to enhanced solar radiation. NH_4^+ has been found to be present at a concentration ~ 4 μM in post monsoon season (Oct-May), which may enter the system from nearby mangrove swamp, sewage discharge point or through benthic regeneration (Pratihary *et al.*, 2009). Nitrogen fixation by *Trichodesmium* that occurs every year starting from late January to May, makes a substantial contribution to the total nutrient budget in the region (Devassy, *et al.,*1978). Following the decay of this bloom, large amount of NH_4^+ (up to 3.3 μM) is released into the medium (Devassy, *et al.*, 1978), which leads to proliferation and succession of other planktonic organisms (Devassy *et al.*, 1979). Further *in situ* measurements of benthic fluxes (Pratihary *et al.*, 2009) have shown that the estuarine sediment is a sink for NO_3^- whereas NH_4^+ remains the dominant N form that is released from sediments in a significant quantity in premonsoon months. Only during the monsoon season (June-Sept) the estuarine waters get enriched with NO_3^- (~8 μM) along with PO_4^{-3} (2.5 μM) and SiO_4^{-4} (>60 μM) (Pratihary, 2008) but even then, NO_3^- remains unutilized, because of cloud cover and turbidity which results in low algal productivity (61.7 mmol C m^{-2} d^{-1}; Ram *et al.*, 2003). Thus, NH_4^+, probably supports the estuarine productivity in non monsoon period.

7A.5: CONCLUSIONS

Nutrient enrichment experiments were carried out to understand the interaction between the phytoplankton growth and nutrient uptake. The results reveal that the estuarine autotrophs were nitrogen limited in the study area during premonsoon period and that the addition of nitrate greatly stimulated the growth leading to biomass accumulation. The presence of considerable amount of NH_4^+ did not have any inhibitory effect on NO_3^- uptake. Rapid uptake of nutrients was observed after a lag phase of 24-32 h and the uptake was significantly dependent on the fast growing diatom taxa and showed high growth rates. *Thalassiosira subtilis* was one of the most sensitive species to low oxygen throughout the incubation period while some species *viz. Melosira* sp, *Rhizosolenia crassispina,* and *Ditylum brightwellii* were found to be initially sensitive to hypoxia (<2 mL O_2 L^{-1}) but their abundance increased after 24 h .

Table: 7A.I: A comparative percentage distribution of dominant pigments in Bottle (**A**), (**B**) and (**C**) at 32 h of incubation period in March (experiment -2)

Percentage distribution of dominant pigments (Experiment-2)				
Time	T0 (initial)		At 32 h	
Pigment	Ambient	Bottle-A	Bottle-B	Bottle-C
Chlorophyll *a*	86	77	66.4	62.5
Chlorophyll $c_1 c_2$	4.4	10	16	16
Chlorophyll *b*	1.3	1	0.3	1.4
Zeaxanthin	2.8	1	1.4	4.4
fucoxanthin	2.9	8	13.6	12.4
Others	2.7	3	2.4	3.3
Total (ng L^{-1})	279	1907	1751	382

Table: 7A. II: Comparative uptake of nitrate and ammonium by estuarine phytoplankton. Exp.1- Bottle (**A**) enriched with NO_3^- and NH_4^+; Bottle (**B**) with nitrate and Bottle (**C**) as control . Exp.2- Bottle (**A**) and (**B**) enriched with only NO_3^-

N-Nutrient	Experiment-1(February)					Experiment -2 (March)				
	Bottle	% Nutrient	% Nutrient utilized	uptake rate (µmol $L^{-1}h^{-1}$)	RPI	Bottle	% Nutrient	% Nutrient utilized	Uptake rate (µmol $L^{-1}h^{-1}$)	RPI
NO_3^-	A	71	84	0.39	1.19	A	93	87	0.78	1.04
NH_4^+	A	27	49	0.01	0.19	A	0.04	14	0.003	0.06
NO_3^-	B	81	65	0.18	1.16	B	84.15	93.9	0.377	1.02
NH_4^+	B	17	30	0.01	0.41	B	14.48	30.58	0.008	0.5
NO_3^-	C	17.7	65.33	0.39	1.172	C	37.63	89.3	0.311	1.18
NH_4^+	C	79.1	30.27	0.033	0.036	C	49.46	6.5	0.02	0.3

Table: 7A.III: Comparative uptake ratio of DIN, DIP and Si by estuarine phytoplankton.

	Experiment-1 (February)		Experiment-2 (March)	
Bottle	DIN/DIP	DIN/Si	DIN/DIP	DIN/Si
A	15.85	0.99	18.78	1.23
B	13.07	0.51	16.20	0.85
C	12.36	0.38	18.29	1.11

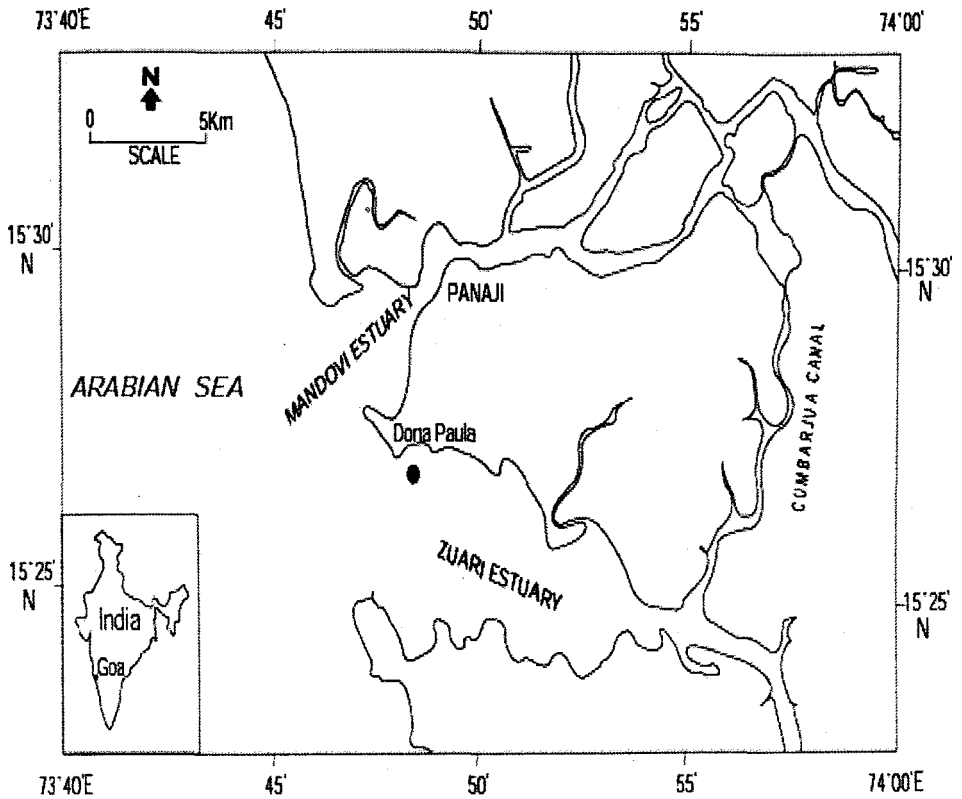


Fig. 7A.1: Map showing study location (solid circle), near the mouth of the Zuari estuary where *in-situ* experiment was conducted.

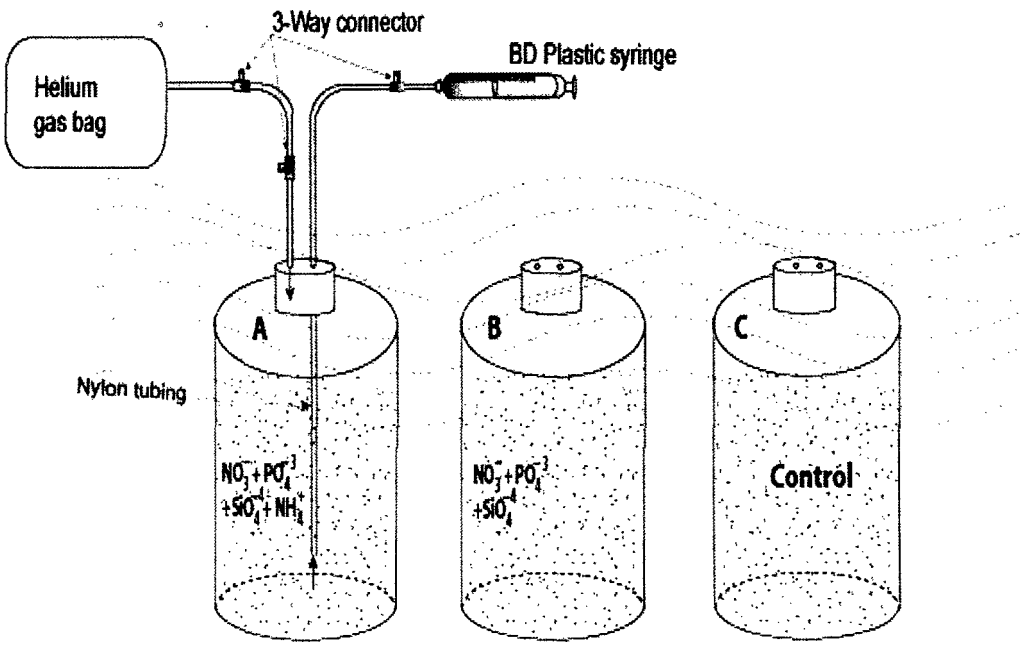


Fig. 7A.2: A schematic diagram of the experimental-1 setup carried out in the Zuari estuary: Bottle (**A**): enriched with NO_3^-, PO_4^{-3}, SiO_4^{-4}, and $NH4^+$; Bottle (**B**): enriched with NO_3^-, PO_4^{-3} and SiO_4^{-4} and Bottle (**C**): acts as a control without any additional nutrients.

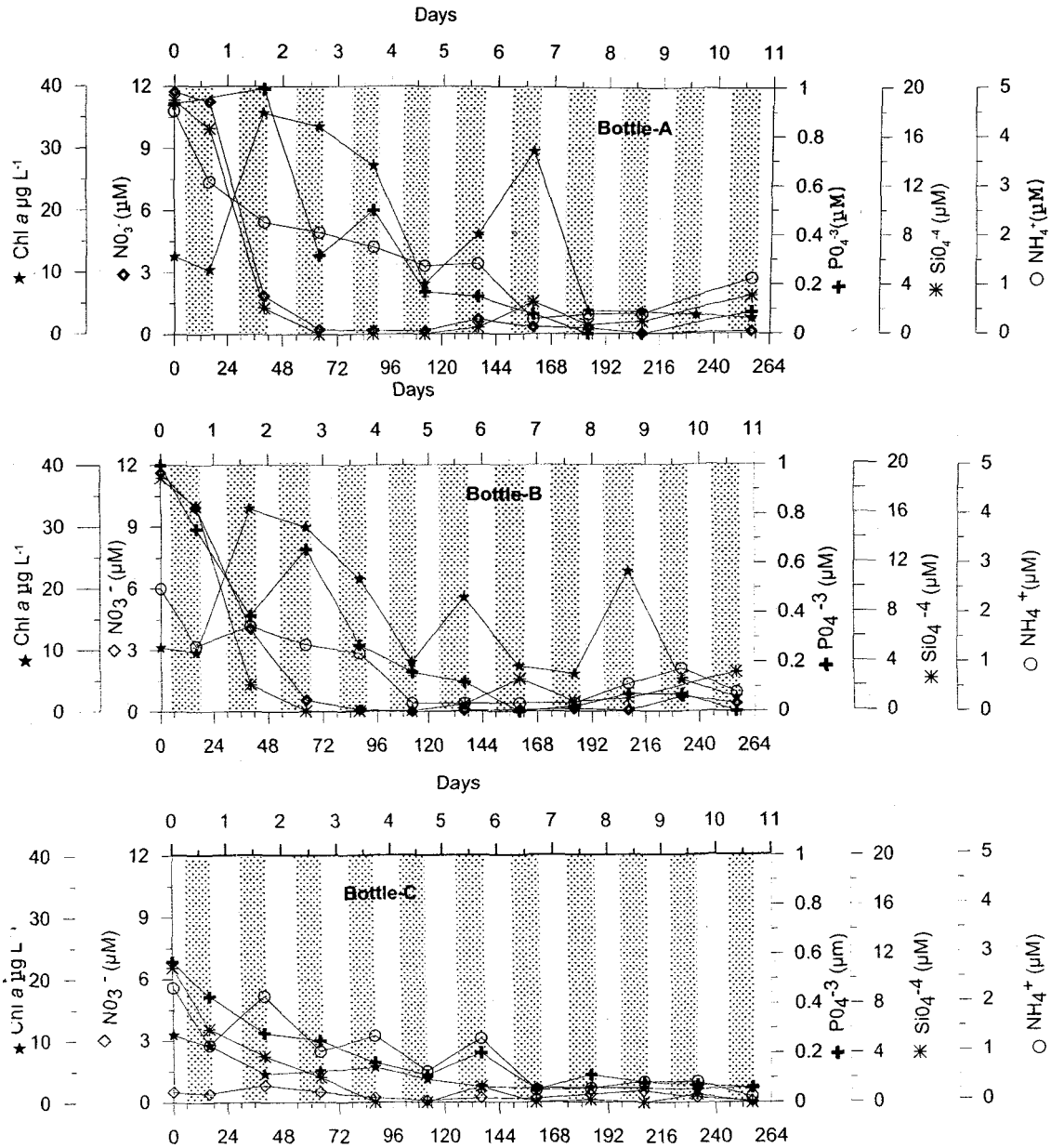


Fig. 7A. 3: Comparative variations in chl *a* and nutrient concentrations with time of incubation in February (experiment-1). Bottle (**A**): NO_3^-, PO_4^{-3}, SiO_4^{-4}, and $NH4^+$; Bottle (**B**): NO_3^-, PO_4^{-3} and SiO_4^{-4}, and Bottle (**C**) without any additional nutrients (control). Shaded portions represent dark periods.

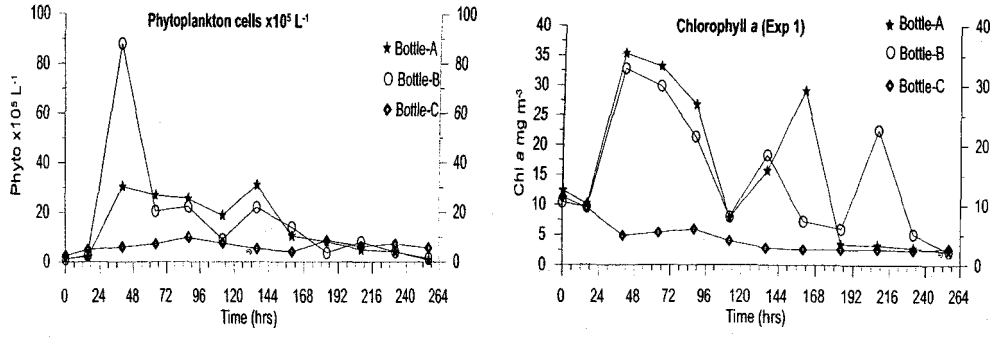


Fig. 7A.4a: Variation in phytoplankton abundance and biomass, in Bottle (**A**) and Bottle (**B**) during February (experiment-1).

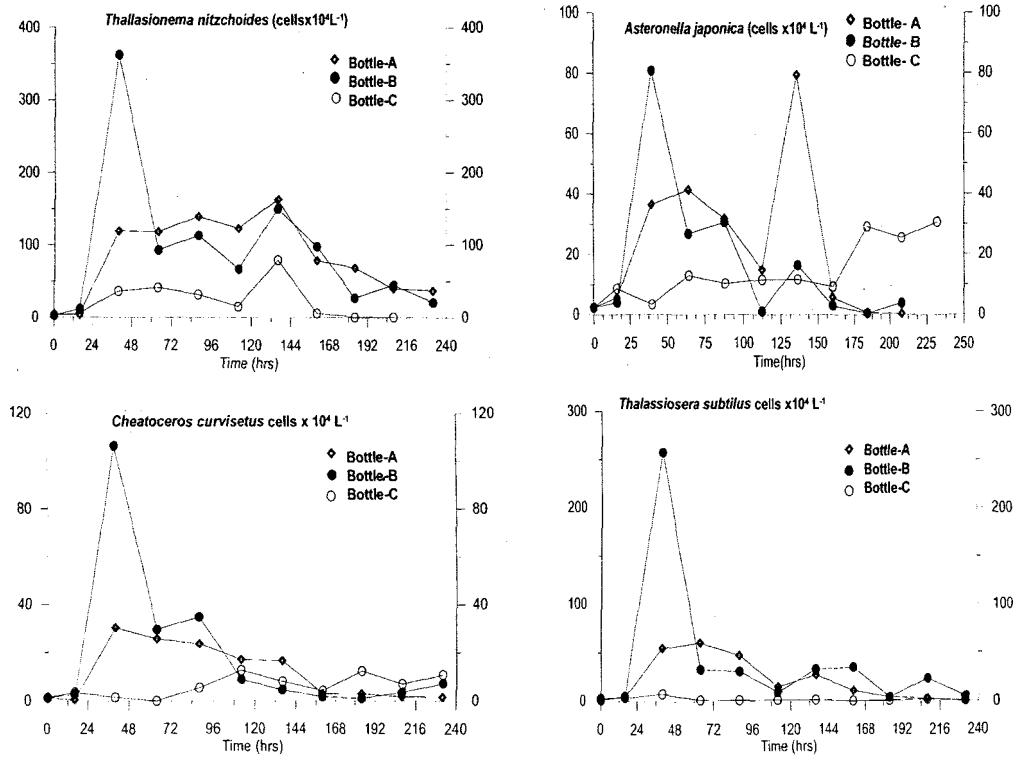


Fig. 7A.4b: Variations in abundance of some diatom species *viz. Thalassionema nitzschioides*, *Asterionella japonica*, *Chaetoceros curvisetus* and *Thalassiosira subtilis* with incubation time in Bottle (**A**), (**B**) and (**C**) during February (experiment-1).

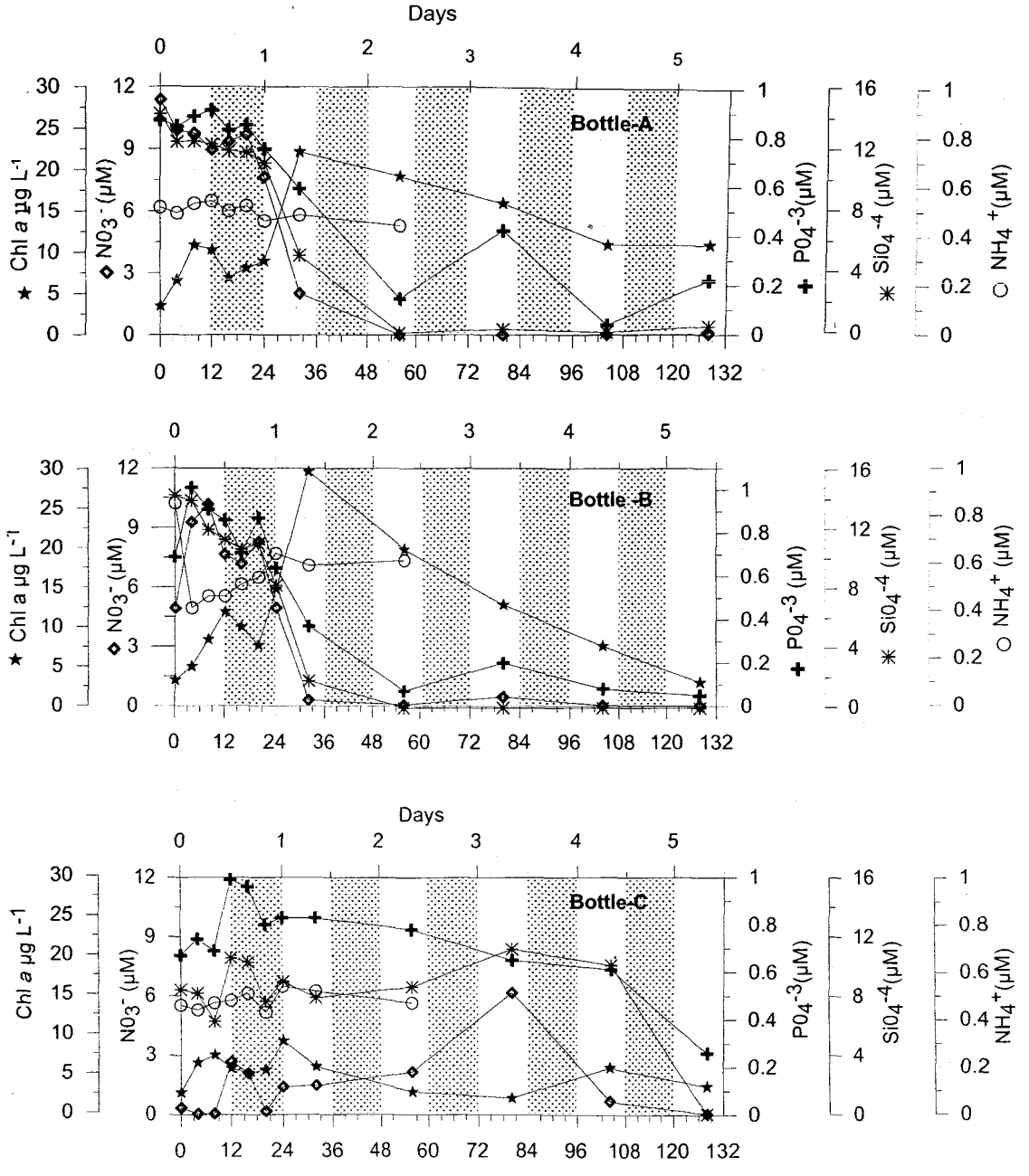


Fig. 7A.5: Comparative variations in chl *a* and nutrient concentrations with time in March (experiment- 2). Bottle (**A**): with added nutrients NO_3^-, PO_4^{-3}, SiO_4^{-4}; Bottle (**B**): with nutrients, as in bottle (A), but deoxygenated (< 2 mL O_2L^{-1}) and Bottle (**C**): without any additional nutrients as control.

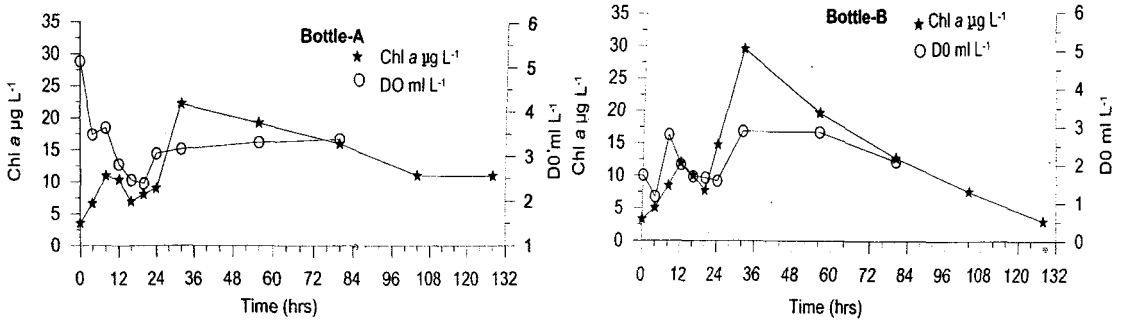


Fig. 7A.6: Co-variations in chlorophyll *a* and dissolved oxygen in Bottle (**A**) and (**B**) in March (experiment- 2).

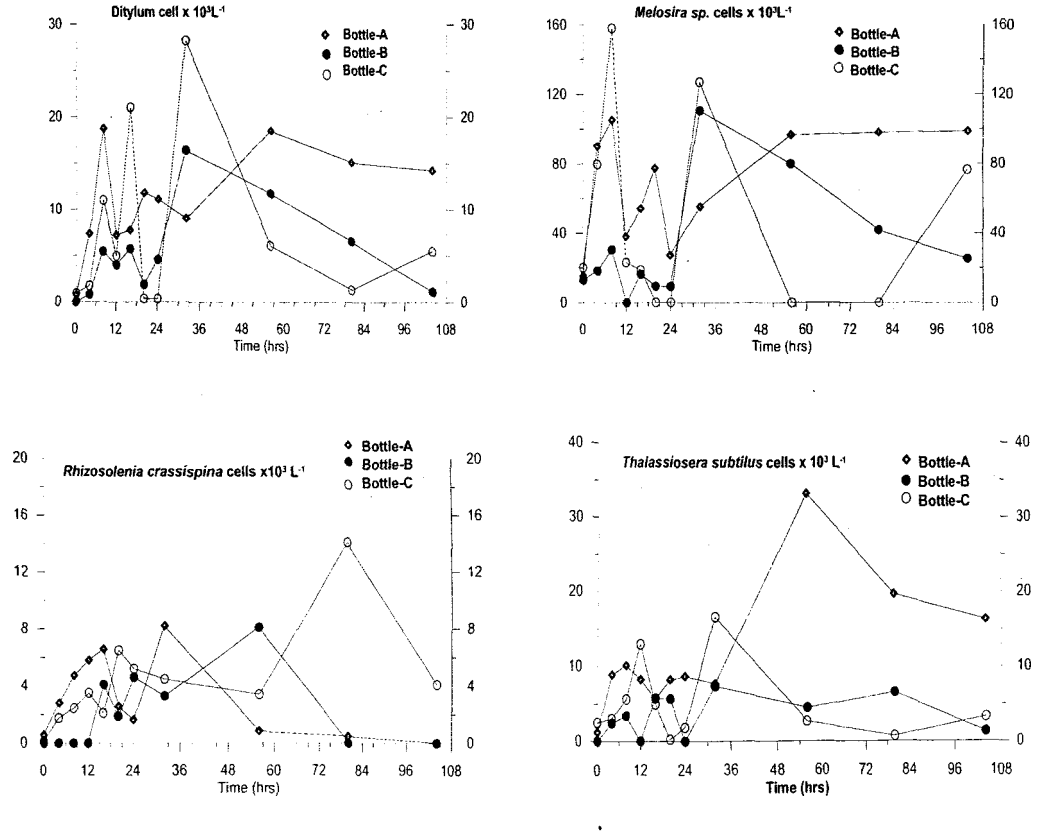


Fig. 7A.7a: Variations in cell abundance of some diatom species *viz. Ditylum brightwellii*; *Melosira* sp; *Rhizosolenia crassispina*; *Thalassiosira subtilis* with incubation time in Bottle (**A**), (**B**) and (**C**) during March (experiment-2).

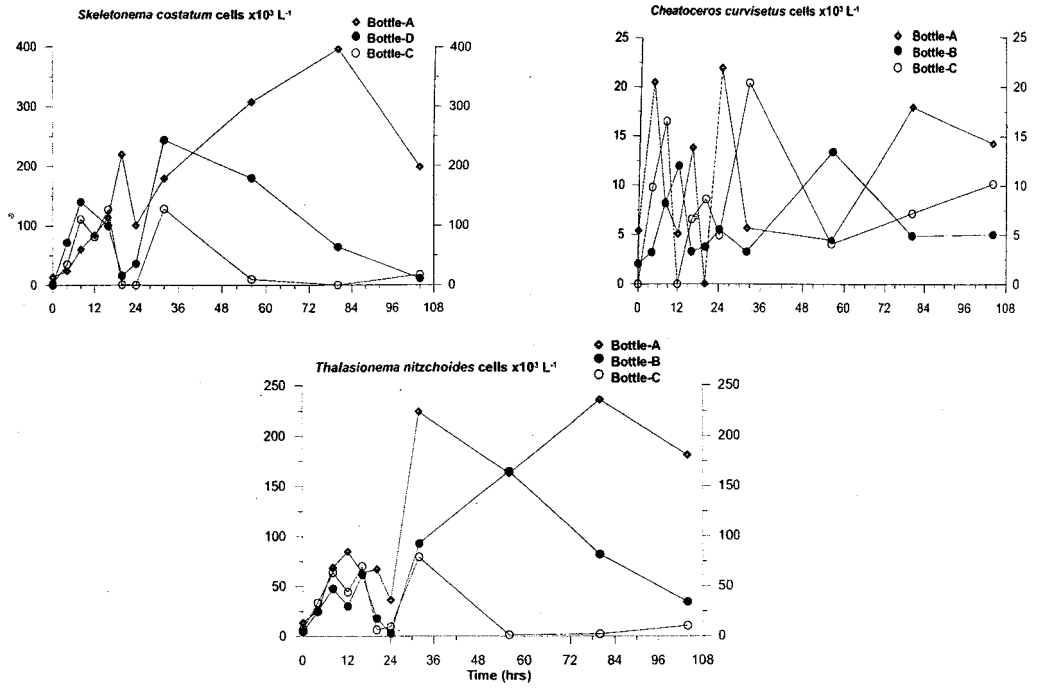


Fig. 7A.7b: Variations in cell abundance of some diatom species *viz. Skeletonema costatum*; *Chaetoceros curvisetus*; *Thalassionema nitzchioides* with incubation time in Bottle **A**, **B** and **C** during March (experiment-2).

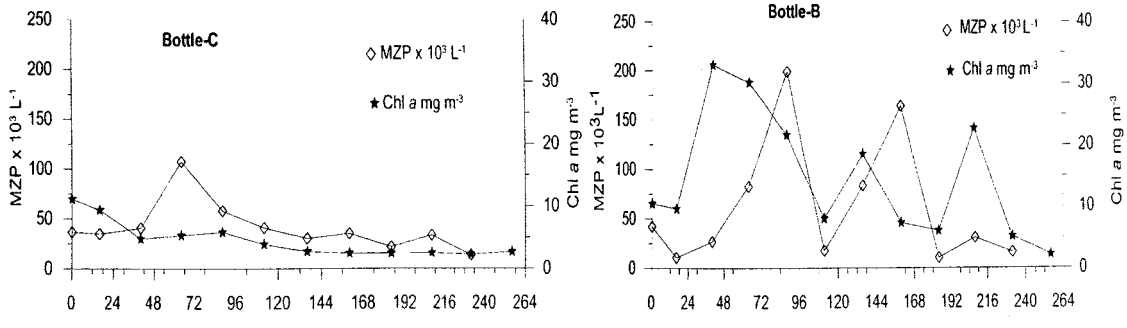


Fig. 7A.8: The grazing effect of microzooplankton on phytoplankton biomass (chl *a*) with time in Bottle (**B**) and Bottle (**C**) control in February (experiment -1).

SECTION-7B

EFFECT OF HYPOXIA ON MESOZOOPLANKTON COMMUNITY IN THE ZUARI ESTUARY: A LABORTORY EXPERIMENT

7B.1: INTRODUCTION

The entire western continental shelf experiences coastal upwelling during the SWM. This effect is also seen extending upto the mouth of Mandovi-Zuari estuary which brings cold nutrient rich but low oxygenated waters into the river mouth (Sankaranarayanan and Jayaraman, 1972). This leads to high primary productivity that in turn supports secondary production (Devassy and Goes, 1988). Dissolved oxygen is an important resource for efficient metabolism (Eckerts, 1983; Hochachka and Somero, 1994) and also influences the distribution and diel migration of zooplankton taxa below the thermocline in the open ocean. Further, due to excess nutrients, phytoplankton growth, organism respiration, death and decomposition of organic matter by microorganism, oxygen is depleted. The combination of low oxygen, high hydrostatic pressure and high organic matter input to the sea floor creates a stressful but food-rich environment for the benthic fauna which are able to tolerate the severe oxygen depletion (Diaz and Rosenberg, 1995; Levin, 2003). But there are no reports or clear evidences stating the fate of zooplankton community thriving in oxygen depleted water in this estuarine system. In this experiment we tried to study the zooplankton community present in the study region and tried to determine the dissolved oxygen threshold on zooplankton by exposing slowly to different oxygen gradient. Thus, a lab based experiment- part I and II was conducted on the zooplankton community (few representative groups) collected from the Zuari Bay in the month of May-June 2009 respectively. This experiment was designed to stimulate the effect of upwelling and study the response of individual organism with low oxygen/stress.

7B.2: METHODOLOGY

7B.2.1: COLLECTION OF MESOZOOPLANKTON

Mesozooplankton samples were collected by horizontally towing using the HT (Heron Tranktor) Net for 5 min. Organisms were collected live taking utmost care so as to avoid damage to the delicate animals. The animals were initially sorted upto group level and later identification upto generic/species level was done under stereozoom microscope by using taxonomic keys.

7B.2.2: EXPERIMENTAL SET UP

The experiment was carried out in a transparent polycarbonate Nalgene bottles (2.5-litre capacity) that were modified by providing three nylon tubes running through the cap of the bottle in addition to a pre-calibrated oxygen sensor (Unisense OX-50, Denmark) for continuous recording of DO (Fig. 7B.1). One of the nylon tubes was used for purging the water with helium gas, whereas the other was used for drawing water samples. The water sample withdrawn was replaced by helium gas from a bag connected to the outlet. The third was used as an outlet for He when the water was degassed. In addition to continuous monitoring with the sensor, DO was also determined by Winkler method manually for discrete samples from time to time following colorimetric method (Pai *et al.*, 1993). Hamilton gas tight syringes were used to draw samples for DO. Each tube was fitted with a three-way valve at its outer end and extreme care was taken to maintain the entire system air tight throughout the experiment. The experimental bottle was placed on a magnetic stirrer to ensure uniform mixing at all times (Fig. 7B.1).

7B.2.3: PREPARATION OF SPECIMEN VIALS

Live zooplankton samples collected by HT net were quickly brought to the laboratory where they were transferred immediately into fresh sea water containing food of mixed phytoplankton culture. After overnight feeding, the animals were shifted to a petri dish and only live and actively swimming organisms were picked up for the experiment. A total of 10-20 such organisms were transferred into 40-ml screw-cap glass vials (Supelco Inc.), the septa of which

had been replaced with a 10 μm mesh to ensure that the organisms were caged and experienced the same conditions as the water outside the vials when exposed to different oxygen gradient. The vial contained enough phytoplankton cells for the animals to feed during the experimental period. Due care was taken not to trap air bubbles in the vials while caging the organisms. The experiment was performed in sets of 3 replicates. A control bottle with the same number of organisms was also maintained along with the experimental bottle and the water in this bottle was continuously aerated. All vials were kept vertically inside the bottle in such a way that the animal motility could be tracked with the naked eye and also continuously through video imaging.

7B.2.4. SAMPLING

Before the experiment, the oxygen sensor was calibrated and readings were recorded. The ambient DO content was measured before the experiment by drawings the water with help of Hamilton glass syringe connected to the 3-way valve. The DO analysis was determined spectrophotometrically as already given in details in chapter 3. The water inside the bottle was then purged with helium to reduce the DO level. This degassing process was done very slowly (ca.1 ml decrease in DO per hr). The response of animals to varying oxygen levels was continuously observed and the threshold DO values were noted for different groups. This threshold corresponded to a complete cessation of mobility and sinking to the bottom. At this point, the experiment was terminated and vials were immediately spiked with vital stain Neutral red (Dressel *et al.*, 1972) to check the viability of the organisms. After 15 min of staining, 2-3 drops of glacial acetic acid were added. The stained organisms were then examined using a dark field microscope with dim illumination. Live animals that took the stain turned a deep magenta hue while dead animals were stained pale red. The experiments were repeated batch-wise at different oxygen concentrations (high to low) as described below.

7B.3: EXPERIMENTAL RESULTS

7B.3.1: EXPERIMENT PART I (MAY 2009)

At the start of the experiment, the DO content of water was ca. 3.8 ml l^{-1} (Fig. 7B.2a). The experiment was initiated by purging the water with helium at a slow, steady rate. This degassing process was done very slowly (ca. 1 ml decrease in DO per hr) until a DO level of ca. 1.5 ml l^{-1} was attained. The DO was continuously monitored with the oxygen sensor. The composition consists of mixed population of few representatives *viz., Evadne tergestina*, copepode nauplii, cyrripede nauplii, *Eucalanus juvenile, Paracalanus parvus, Pseudodiaoptomus aurivelli, Acartia* spp., *Labidocera pectinate, Tortanus forcipatus, Metacalanus, Oithona* spp and *Corycaceus* sp. As mentioned earlier, only active and well-fed organisms were used in the experiment. Mobility of organism in the glass vials was visually found to decrease as the oxygen concentration dropped to 1 ml l^{-1}. At 0.84 ml DO l^{-1} Cladocera (*Evadne tergestina*) and Decapode larvae (*Lucifer hensani, Lucifer* sp., brachyuran zoea) and Copepod (*Eucalanus subcrassus*) were found dead, as also confirmed through viability stain - Neutral red. Similar experiment was conducted several sets with enough replicates to confirm the same.

Based on the results of the first experiment, the second set of experiments was to focus on organisms' response to DO levels below 1 ml l^{-1}. Here, organisms found susceptible to DO ~0.8 ml l^{-1} such as decapods larvae were not included. It was further confirmed that copepod species (*Paracalanus parvus, Pseudodiaoptomus aurivilli, Acartia* sp., *Labidocera pectinate, Tortanus forcipatus, Oithona rigida, Corycaceus* sp., and *Metacalanus aurivilli*) were live even up to 0.8ml DO L^{-1} but mortality occurred below 0.8ml L^{-1} *i.e.* at 0.72 ml DO l^{-1}. Interestingly, larval forms (nauplii) were still found to be visually active at this concentration; this was confirmed by staining. Therefore, a third set of experiment was carried with only copepod nauplii and cirripede nauplii. These organisms exhibited a high degree of tolerance to oxygen depletion i.e. they remained alive even at a DO level of 0.6 ml l^{-1} (Fig. 7B.2a). However, further reduction of DO to 0.21 ml l^{-1} led to mortality of these organisms.

7B.3.2: EXPERIMENT PART-II (JUNE, 2009)

This experiment was conducted in the month of June, 2009. The zooplankton composition remained the same as mentioned in Experiment-1, however, this experiment also with few polycheates and harpacticoids as these were predominant in samples at this time of the year. The oxygen conc. ranged from (0.8 - 0.108) O_2 ml L^{-1}. The results of this experiment were consistent with those of the third experiment of part I, wherein cirrepede and copepod nauplii did not survive below 0.6 ml DO l^{-1} (Fig. 7B.2b). On this occasion, the viability checked at DO content of 0.49 ml l^{-1} confirmed the mortality of harpacticoid copepod (*Euterpina acutifrons*), cirripede and copepod nauplii. Of all the animals that were exposed to low oxygen, only juveniles of the polycheate, *Staurocephalus* sp. were found to survive at DO concentration of ~0.1 ml l^{-1}.

7B.4: DISCUSSION

Life requires oxygen to sustain its metabolic processes. In the aquatic bodies it is supplied through photosynthesis and from the atmosphere. And conversely oxygen is depleted during organism respiration and by decomposition of organic matter.

Acute oxygen deficiency is experienced during summer upwelling along the western continental shelf (Naqvi *et al.*, 2000, 2006) covering a stretch of 180,000 km^2 coast line. This effect is also seen extending upto the mouth of Mandovi-Zuari estuary (Sankaranarayanan and Jayaraman, 1972). Thus, bottom waters of coastal upwelling regions are frequently exposed to hypoxia, suboxia or anoxia leading to depletion of marine animals in the affected regions. This has also been known to have a profound impact on benthic fauna (Ingole *et al.*, 2010) found in the depths where the oxygen content of bottom water was less that 0.5ml/L (Carruthers *et al.*, (1959) The development of these conditions represents an acute perturbation to ecological dynamics and fisheries. Although the impact of hypoxia on benthic communities has been studied intensively, less is known about its effect on pelagic communities in coastal ecosystems. Water column hypoxia is known to alter the development (e.g. physiology and life

cycle), recruitment, patterns of species distribution and migration (Ekau *et al.*, 2009). But there are no reports or clear evidences stating the fate of zooplankton community thriving in oxygen depleted water in this estuary unlike some previous studies (Roman et al., 1993) in the Chesapeake where a submersible pump was used for zooplankton sampling from low oxygen waters. The study highlights the experiment carried out in laboratory at different oxygen concentrations and its effect particularly zooplankton community thriving in oxygen stress condition in the bottom water of Zuari estuary. The ability of certain zooplankton species to transit through or even live in low oxygen environments is apparently linked to the presence and activity of enzymes of the anaerobic metabolism *i.e.* lipid dehydrogenase (LDH) (Escribano, 2006). For example, the LDH activity is known to remain high in *Euphausid* sp. living in the OMZ (Gonzalez and Quiñones, 2002). Nonetheless, previous observations show that hypoxia tolerance and threshold values are species and stage-specific and can vary widely (Miller *et al.*, 2002). Organisms such as *Paracalanus* spp., *Acartia* spp., *Centropages* spp., *Evadne tergestina, Lucifer hensani, Oikopleura* sp. and Decapods are predominant species found during the SW monsoon period is been concluded from the field data. Results of our experiments involving copepods such as *Paracalanus parvus, Acartia* sp., *Labidocera pectinate, Oithona rigida, Corycaceus* sp. and *Euterpina acutifrons* that showed tolerance to low oxygen are consistent with data on their actual occurrence in low oxygen waters. Few typical estuarine species (*Pseudodiaoptomus aurivilli, Tortanus forcipatus* and *Metacalanus aurivilli*) were also found to be associated with hypoxia. While, decapod larvae appear to be quite vulnerable to oxygen deficiency. Polychaetes are usually the most abundant taxon in benthic communities and have been most often utilized as indicator species of environmental conditions (Shivadas *et al.*, 2010). Our experimental results show that the larval forms of cirrepede could survive at oxygen level as low as ~0.5 ml l^{-1}, and the polycheate *Staurocephalus* sp. exhibited an even greater tolerance to oxygen depletion in water. It is also possible that this polychaete has a low metabolism and therefore requires less oxygen as also seen in some copepods (Lampitt and Gamble, 1982). Antioxidant enzymes possibly enable these organisms to adapt to the oxygen stress, a mechanism seen in harpacticoid, *Tigriopus japonicus* (Lee *et al.* 2007; Raisuddin *et al.* 2007) and molluscs (Viarengo *et al.*, 2007). Recent work by Desai and Prakash (2009) has shown that the cirrepede nauplii (*Balanus amphitrite*) also produce antioxidant enzymes to overcome acute hypoxia stress.

7B.5: CONCLUSIONS

The zooplankton biomass is generally higher in oxic waters as compared to oxygen-deficient waters. Among various zooplankton, few species of copepods can survive at low concentrations of oxygen (< 1 ml L^{-1}), as revealed by results of laboratory experiments. Larval stages of Cirripede, copepod and polycheate were found to be more tolerant to oxygen deficiency. The mechanism of survival of these organisms in very low oxygen environments is not fully known and is a subject of future investigation.

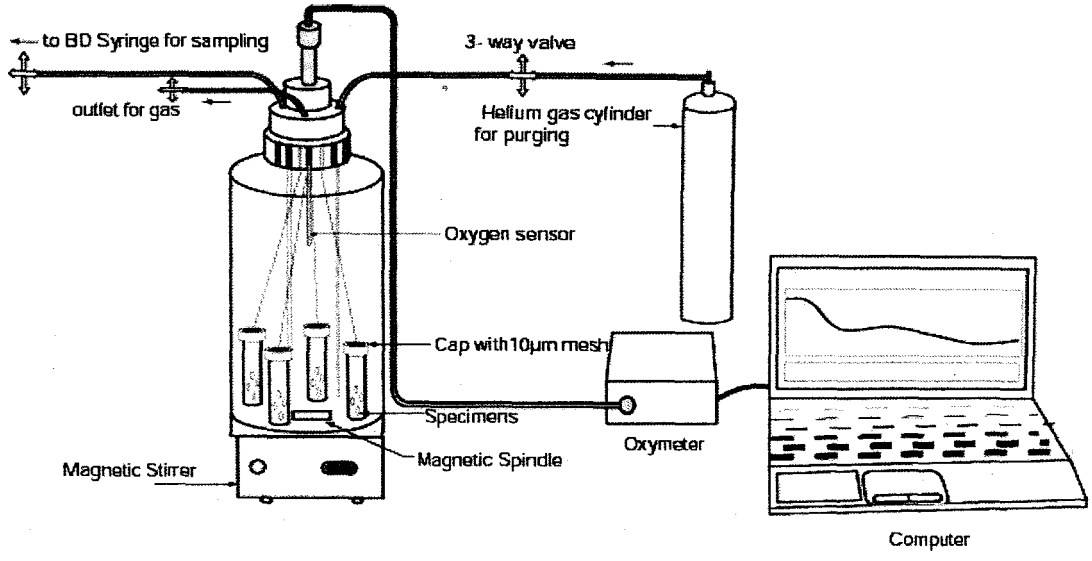


Fig. 7B.1: The experimental set-up showing organisms caged in specimen vials and placed in an air tight container. The lid of the container is fitted with an oxygen sensor and tubes for purging the water with helium and for drawing water samples.

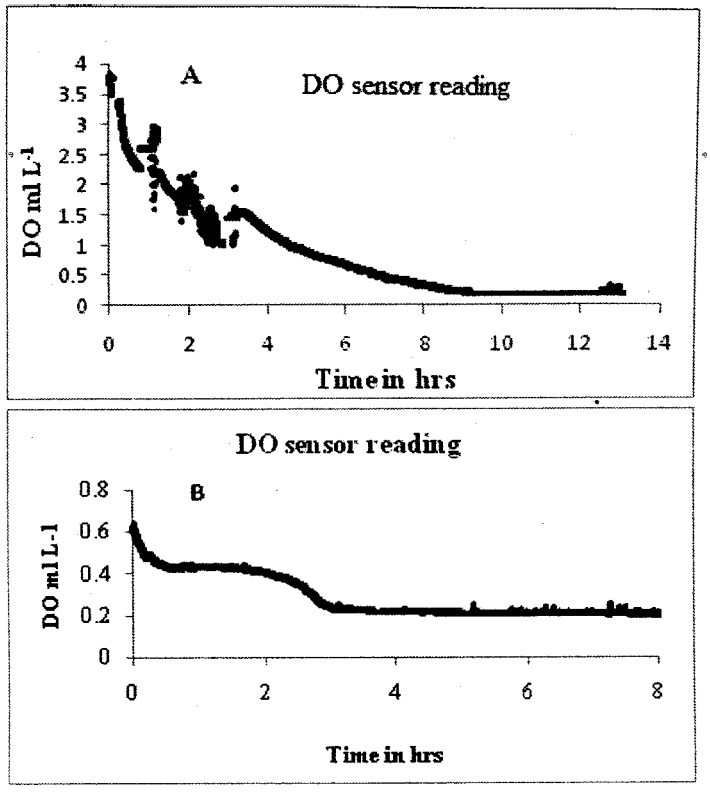


Fig. 7B.2: Plot showing continuous measurements of dissolved oxygen using a DO sensor: first experiment (**a**) carried out for 12 hours beginning with 3.8ml l^{-1} (oxic) to 1ml l^{-1} (hypoxic) level. Second experiment (**b**) carried out for 8 hours beginning with 0.7ml l^{-1} (hypoxic) to 0.2ml l^{-1} (suboxic) level.

CHAPTER 8

CONCLUSIONS AND PERSPECTIVES

The northern Indian Ocean is very different from the other ocean basins in terms of its climatic and oceanographic processes. It is strongly influenced by intense, annually reversing monsoon winds. This is due to the differential heating and cooling of the land and sea. It is the only oceanic region that is bounded by land mass at low latitudes (ca. 25°N). Ensuing wind driven current system of this region causes upwelling thereby increasing the primary productivity of the area. The Indian Ocean, in general, is one of the most productive amoung the world's oceans. It is mainly through the process of increased production levels in the water column. Oceanic subsurface waters and the coastal shelf waters of the eastern Arabian Sea are typical as waters of this region are severely stressed by either perennial/seasonal hypoxia, popularly known as the 'dead zones'. Such characteristic ecosystems are known to have drastic reduction in pelagic and benthic invertebrate communities and fishery landings. Thus, work carried out as a part of this thesis is considered to be important as hypoxia is known to have affected on large coastal ecosystems around the world.

I believe that the overall purpose of the work presented in this thesis has been achieved successfully on several levels. The Ph.D thesis analyses effects of low oxygen on natural plankton communities (phytoplankton and mesozooplankton) of the Arabian Sea. This was addressed through field and experimental studies. Independent mesocosm experiments and a series of laboratory experiments performed in coastal waters of the eastern Arabian Sea. In this thesis, the focus is laid on the differences in plankton between permanent offshore and seasonal coastal hypoxia systems, to reveal indirect effects and interactions with the abiotic environment, which are in general understudied. Biological studies of species composition, abundances, biomass and basic ecological understanding remains very incomplete without this kind of measurements. The salient findings of this thesis have been presented below.

During January to May (pre-monsoon period) and November-December the water column of the study region remains well oxygenated (3.7-4.6 ml L^{-1}). On the onset of southwest monsoon, upwelling processes and land derived nutrients increases surface productivity with

chlorophyll *a* as high as 8.5 mg m^{-3}. Enhanced oxygen consumption by decomposition of organic matter leads eventually to oxygen deficiency in the water column. Subsurface and bottom water becomes suboxic (O_2<0.2ml L^{-1}) between August-September and anoxia (O_2<0.02ml L^{-1}) prevails in October-November.

The studies revealed huge differences in plankton community composition and hereby function of the food web in offshore towards coastal system. Phytoplankton biomass (chl *a*) showed decreasing trend towards the offshore stations along the transect. Diatom diversity was 2.5 fold higher than that of dinoflagellates. Overall, centric diatom dominated (ca. 64%) over the pennate forms.

The prevalence of severe oxygen deficiency in sub-pycnocline waters during senescence phase of the southwest monsoon strongly influences the dynamics of both phyto- and zoo-plankton. While microplakton (mostly diatoms but also dinoflagellates) constitute the major part of the phytoplankton biomass in oxic waters. Diatoms such as *Thalassionema nitzschioides, Pseudo-nitzschia* spp, *Thalassiothrix* sp, *Asterionella japonica, Navicula* spp, *Pleurosigma* spp (pennate forms); *Leptocylindrus minimus, Rhizosolenia setigera,* and *Actinoptichus undulates* (centric forms) were found to be coupled with oxygen deficient waters.

The size fractionated chl *a* measurement study indicates that picoplankton (0.7-5um; Synechococcus) form a large fraction (65-73%) of the total phytoplankton community. Spatially smaller cell size fraction of chlorophyll decreases gradually towards off coast and is taken over by the larger forms of phytoplankton (20-200um). Seasonal studies also state that pico autotrophs (<5-0.7 μm) predominate in oxygen deficient waters prevailing during late southwest monsoon.

Oxygen deficiency in subsurface coastal waters was found to affect the primary production rates particularly during late southwest monsoon.

Copepods were the dominant forms accounting to approx. 65% of the total zooplankton community throughout the year. Higher biomass of zooplankton was reported near coast similar to phytoplankton. Interestingly, study region is largly dominated by carnivores/omivorous forms.

Paracalanus and *Eucalanus* spp were the key species of the coastal region. The zooplankton biomass is generally higher in oxic waters as compared to oxygen-deficient waters. Among various zooplankton, several species of copepods can survive at low concentrations of oxygen (< 1 ml l^{-1}), as revealed by both field data and results of laboratory experiments. Field study discovered dominance of *Acartia amboinensis, Acartia spinicauda, Centropages trispinosus* and other *Centropages* spp in low oxygen shelf waters of India. Laboratory experimental studies show that cirripede and copepod and polycheate larvae are even more tolerant to oxygen deficiency than the adults. The mechanism of survival of these organisms in very low oxygen environments is not fully known and should be investigated in future. *Pleuromama* spp, *Mormonilla* spp, *Rhincalanus* spp, *Eucalanus* spp, *Lucicutia* spp, *Heterorabdus* spp were found to be resident copepod zooplankton in the core of the OMZ in the Arabian Sea.

This study also reports instance of symbiotic association in the phytoplankton of the Arabian Sea (coastal and open waters). Associations were found higher in the open waters than in the coastal region. This study show that in the study area such associations possibly occurring regularly particularly during spring and fall intermonsoon when system undergo oligotrophy. However, the quantitative importance of this association, on the context of oceanic N cycling remains a subject of future research. Thus, such associations need to be closely monitored in aquatic systems with respect to biogeochemistry of the region.

Nutrient enrichment experiments reveal that the estuarine autotrophs were nitrogen limited in the study area during premonsoon period. The addition of nitrate greatly stimulated the growth leading to biomass accumulation. Rapid uptake of nutrients was observed after a lag phase of 24-32 h. The presence of considerable amount of NH_4^+ did not found to have any inhibitory effect on NO_3^- uptake. *Thalassiosira subtilis* was one of the most sensitive species to low oxygen while some species *viz. Melosira* sp, *Rhizosolenia crasstspina* and *Ditylum brightwellii* were found to acclimate to hypoxia (<2 ml O_2 L^{-1}) only after 24 h of exposure .

LEGENDS TO PLATES

Plate I-VII: Photographs of some phytoplankton species of the Northern Arabian Sea

PLATE-I

a. *Thalassiosera subtilus*
b. *Stephanophysis turris*
c. *Guinardiella* sp.
d. *Rhizosolenia setigera*
e. *Rhizosolenia imbricata*
f. *Rhizosolenia robusta*
g. *Planktoniella sol*
h. *Gossleriella tropica*

PLATE-II

i. *Coscindiscus marginatus*
j. *Hyalodiscus stelliger*
k. *Coscinodiscus radiatus*
l. *Corethron hystrix*
m. *Guinardia flaccida*
n. *Schroderella delicatula*
o. *Melosira* sp
p. *Melosira nummuloides*

PLATE-III

q. *Hemiaulus hauckii*
r. *Climacodinium frauenfeldianum*
s. *Chaetoceros messanensis*
t. *Bacteriastrum hyalinum*
u. *Chaetoceros loranzianus*
v. *Chaetoceros curvisetus*
w. *Chaetoceros diversus*
x. *Bacillaria paradoxa*

PLATE-IV

y. Navicula distans

z. Navicula transitans var.derasa

aa. Pleurosigma elongatum

ab. Nitzschia closterium

ac. Coconeis sp

ad. Pseudonitszchia seriata

ae. Meuniera membranacea

af. Thalassionema nitzchoides

PLATE-V

ag. Asterionella japonica

ah. Protoperidinium steinii

ai. Protoperidinium divergens

aj. Protoperidinium conicum

ak. Oxytoxum sp

al. Gonyaulax pacifica

am. Pyrocytis lunula

an. Pyrophacus steinii

PLATE VI

ao. Ceratium furca

ap. Ceratium pentagonum

aq. Ceratium fusus

ar. Ceratium trichoceros

as. Ceratium lineatum

at. Ceratium macroceros

au. Ceratium contrarium

av. Prorocentrum micans

PLATE-VII

aw. Ceratocorys horrida

ax. Ornithocercus thumii

ay. Phalacroma rotundatum

az. Phalacroma cuneus

ba. Dinophysis caudate

bb. Dinophysis miles

bc. Oxytoxum parvum

bd. Oxytoxum namum

PLATE-VIII

be. Gymnodinium

bf. Gymnodinium breve

bg. Balechina coerulea.

bh. Warnowia polyphemus

Plate VII-XI: Photographs of some copepod species from the coastal waters of west coast of India, CaTS site.

PLATE VIII-IX

a. Centropages tenuiremis

b. Centropages calaninus

c. Centropage furcatus

d. Centropages alcocki

e. Calanopia elliptica

f. Acartia erythraea

g. Acartia amboinensis

h. Acartia negligens

PLATE-X

i. Canthocalanus pauper

j. Undinula vulgaris

k. Temora discaudata

l. Tortanus forcipatus

m. Subeucalanus pileatus

n. Eucalanus monachus

o. Lucicutia flavicornis

p. Scolecithrix sp.

PLATE-XI

q. Candacia bradyi

r. Candacia ethiopica

s. Labidocera acuta

t. Labidocera pectinata

u. Labidocera sp.

v. Canalopia minor

w. Euchaeta marina

x. Acrocalanus gibber

Plate XI - XV: Photographs of some epipelagic copepod species of the open waters of Northern Arabian Sea .

PLATE-XII

y. Pleuromamma indica

z. Pleuromama quadrungulata

aa. Pleuromamma gracilis

ab. Pleuromamma xiphias

ac. Metridia princeps

ad. Metridia brevicauda

ae. Aetideus armatus

af. Sappharina metallina

P LATE-XIII

ag. Gaetanus miles

ah. Scolecithrix ctenopus

ai. Euchirella amoena

a.j Euchirella maxima

ak. Scaphocalanus sp

al. Eueugaptilus hectitus

am. Scottocalanus helenae

an. Gaetanus minor

PLATE XIV

ao. Eucalanus elongates

ap. Pareucalanus attenuatus

aq. Rhincalanus nasutus

ar. Rhincalanus rostifrons

as. Arietellus plumifer

at. Aetideus armature

au. Euaetideus giesbrechti

av. Halioptilus longicornis

PLATE-XV

aw. Oncea venusta

ax. Conaea gracilis

ay. Lubbockia squilamana

az. Vettoria sp

ba. Corissa sp

bb. Oncea sp

bc. Aegisthus mucronatus

bd. Saphirella tropica

Plate XVI: Photographs /Dark field images of some copepod species

PLATE –XVI (DARK FIELD IMAGES)

be. Paracalanus aculeatus

bf. Paracalanus parvus

bg. Cosmocalanus darwini

bh. Subeucalanus subtenuis

bi. Eucheata wolfendini

bj. Sapphirina sp.

bk. Euterpina acutifrons

bl. Oithona rigida

Plate XVII: Photographs of various groups of zooplankton community

PLATE-XVII

bm. Sagita enflata

bn. Lucifer hensani

bo. Ostracoda

bp. Euphausia sp.

bq. Solmundella bitentaculata

br. Oikiopleura doika.

bs. Staurocephalus sp (juvenile)

bt. Fish larvae

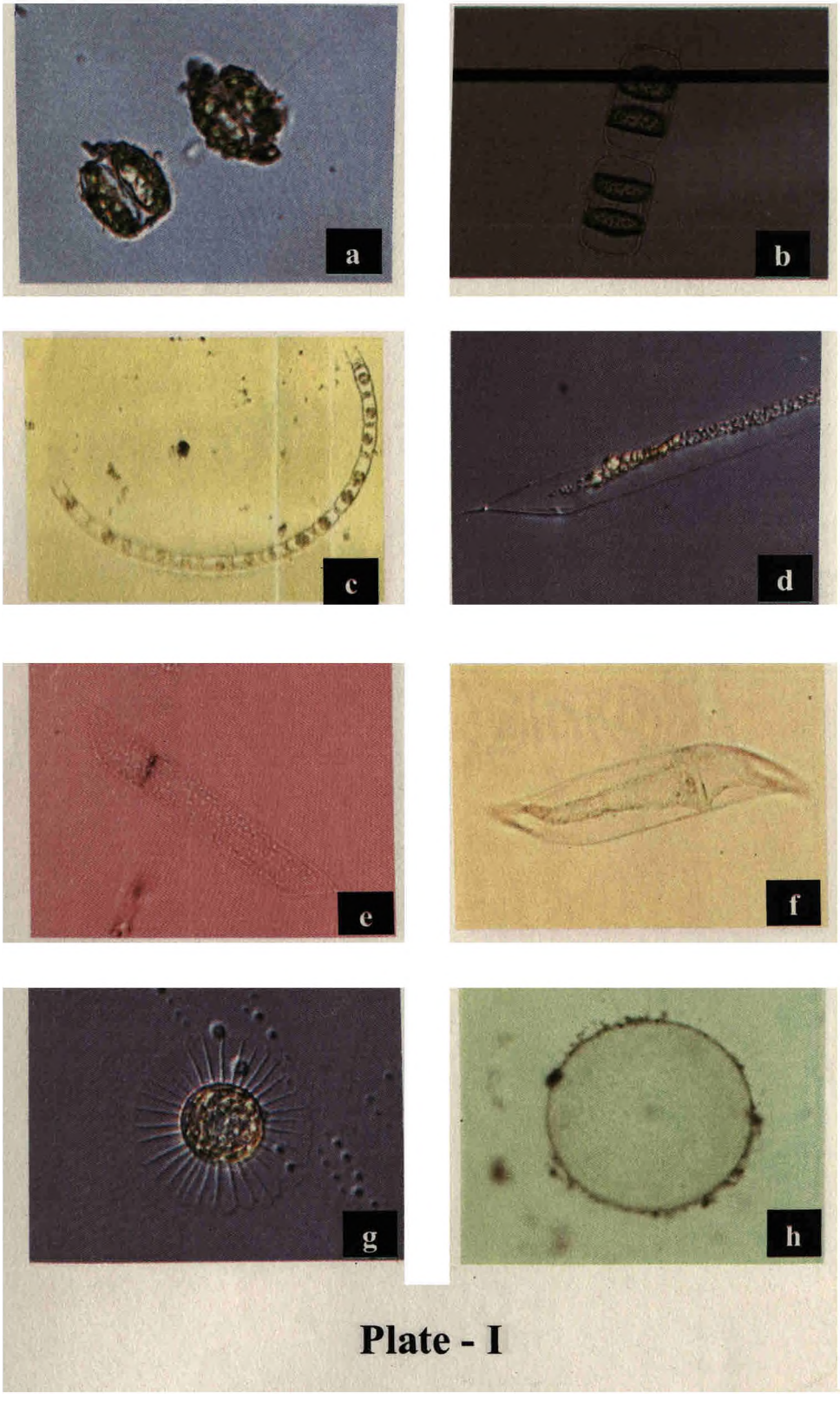
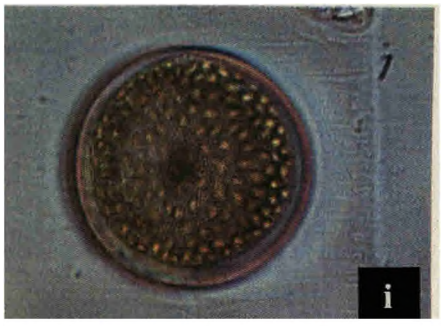
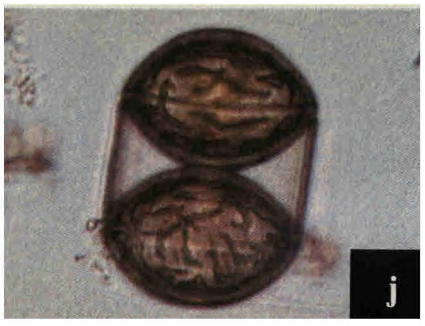
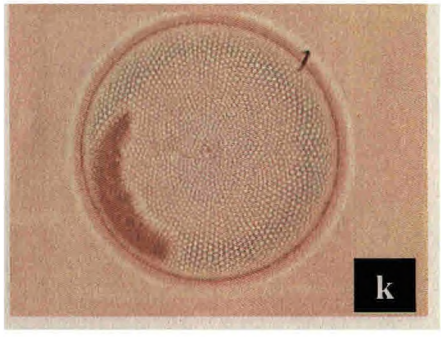
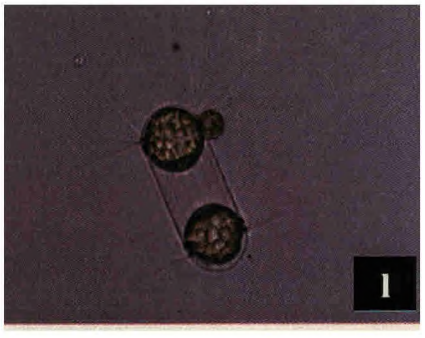


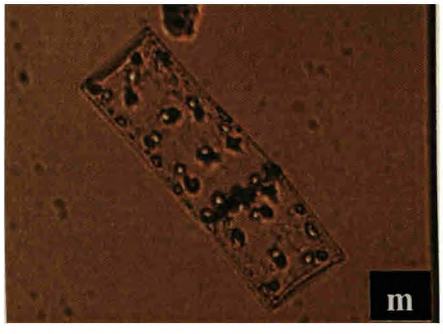
Plate - I

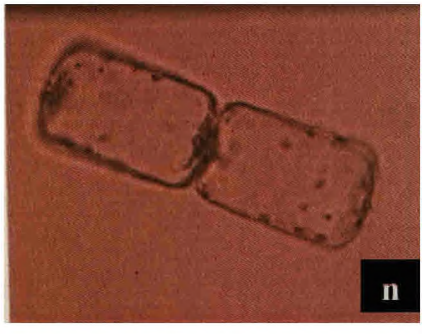












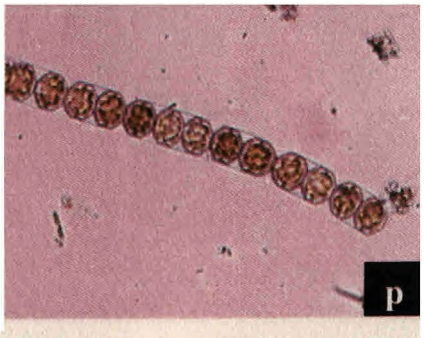
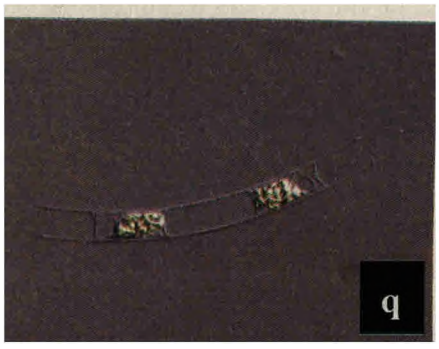
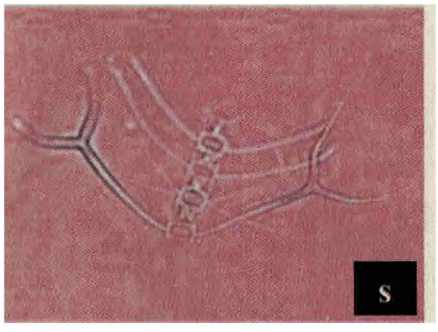
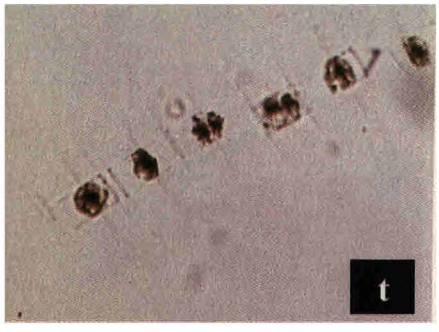
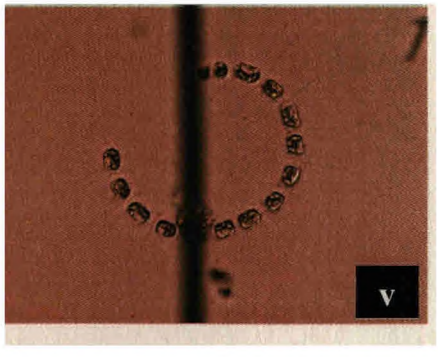


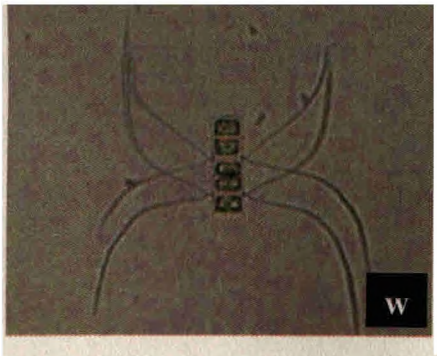
Plate - II

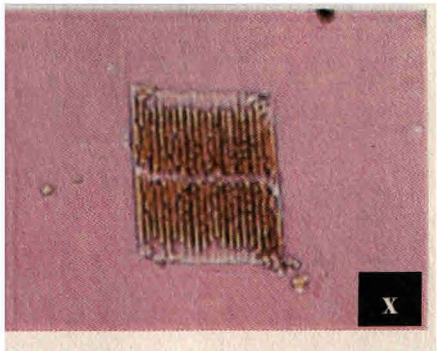












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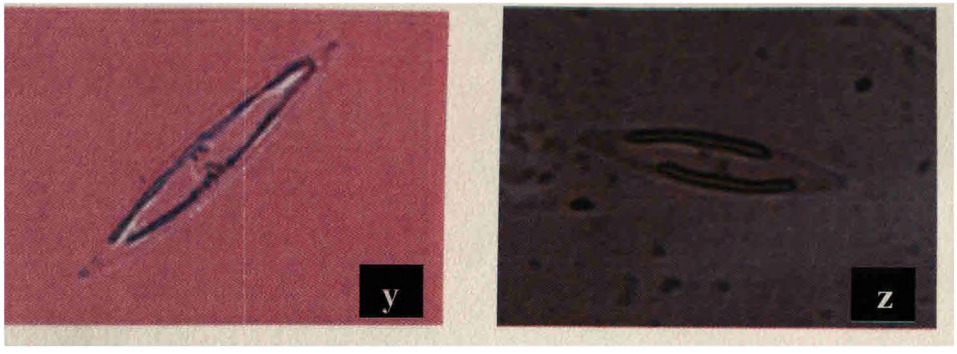

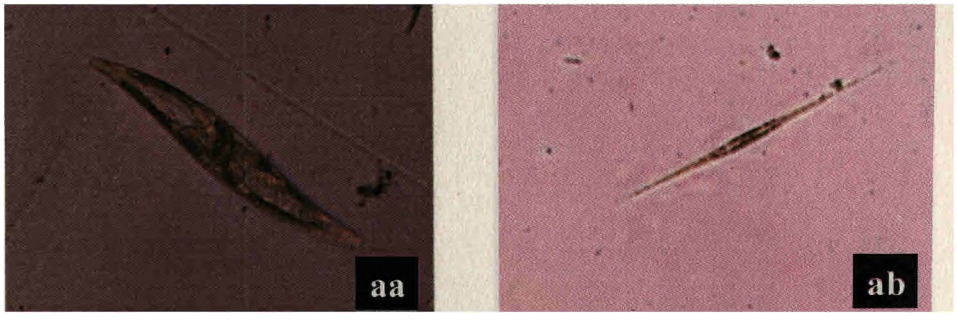

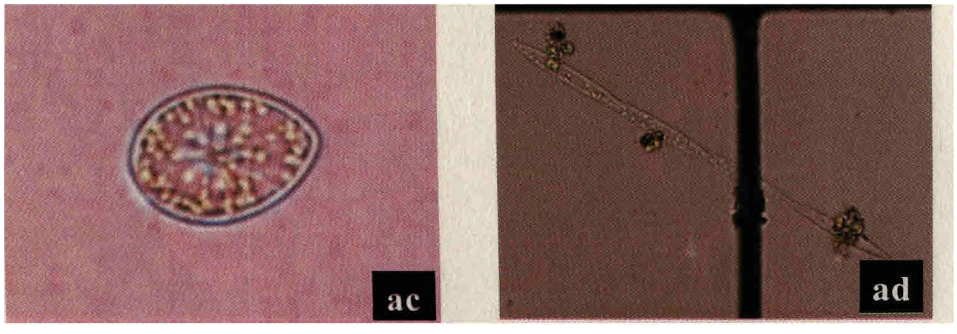

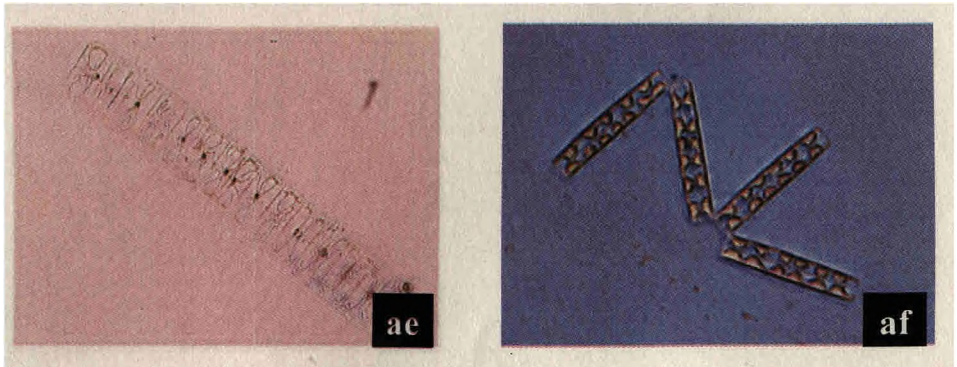

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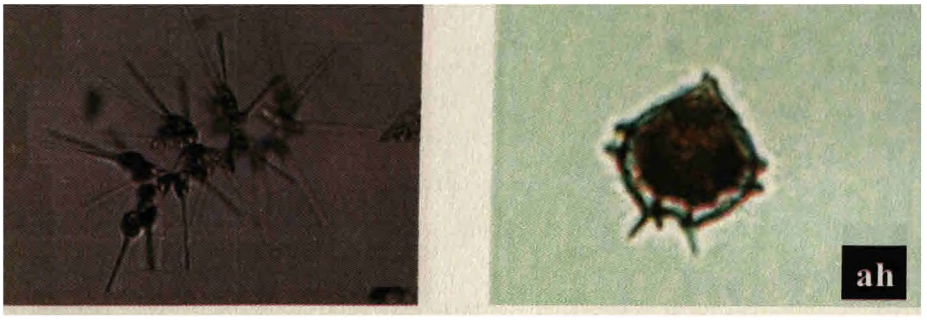

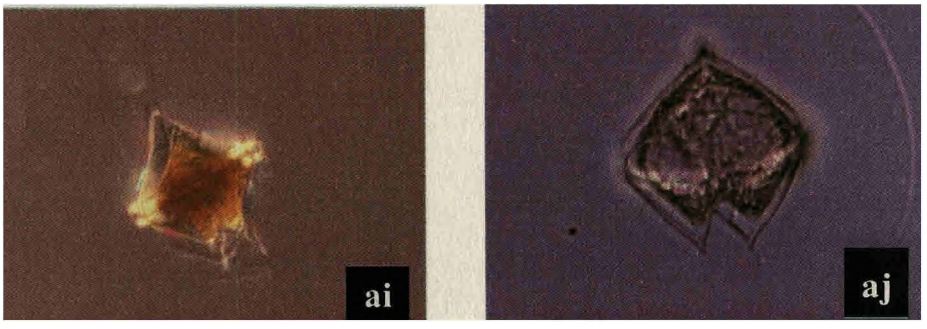

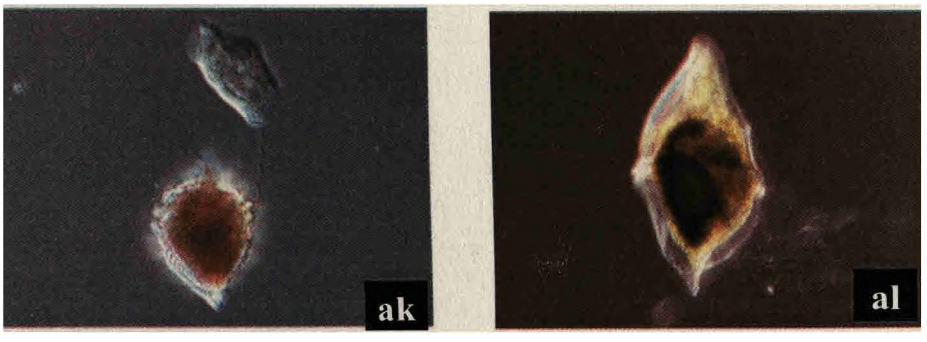

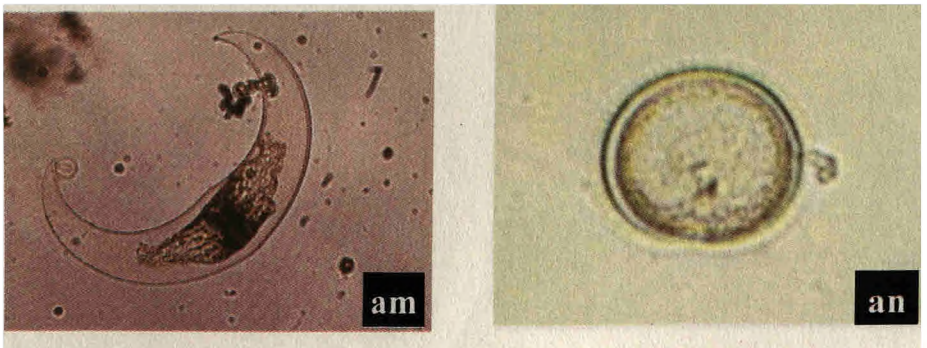

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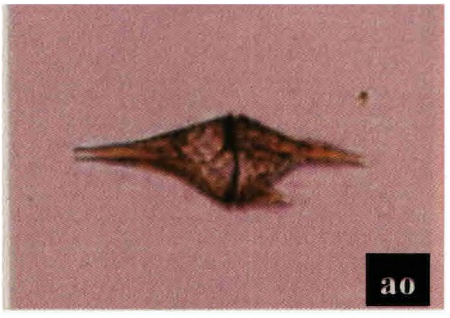

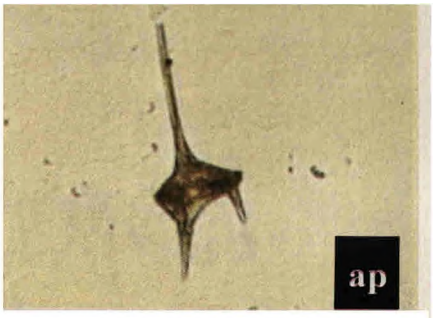

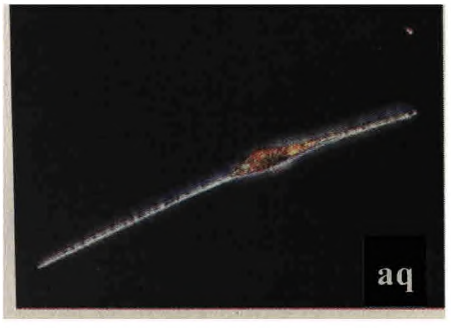

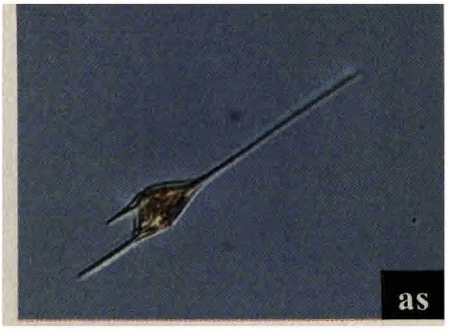

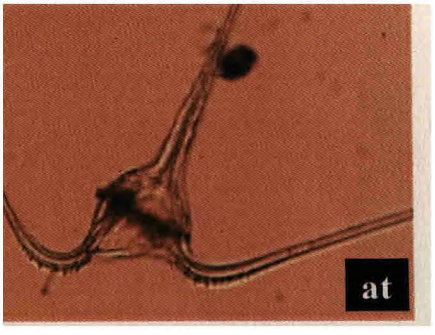

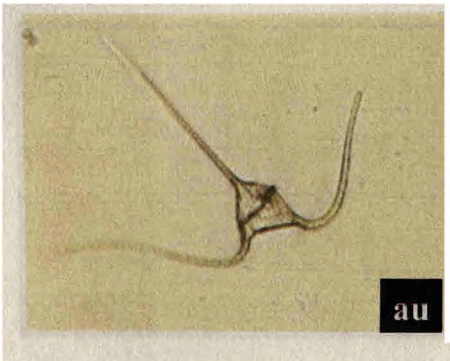

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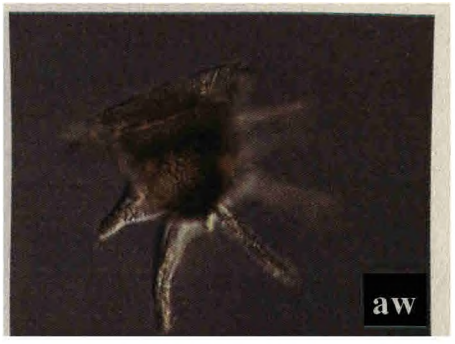
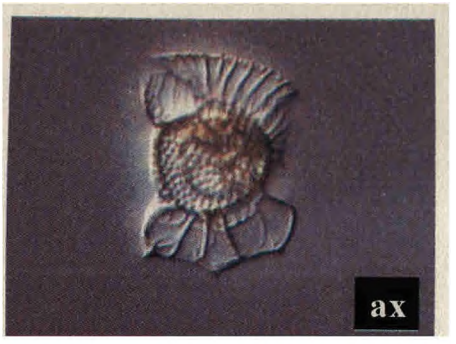
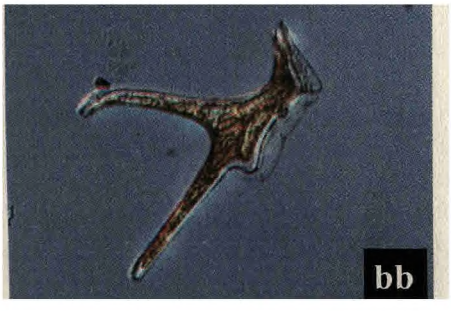






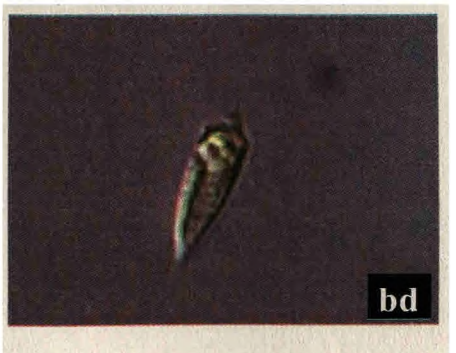
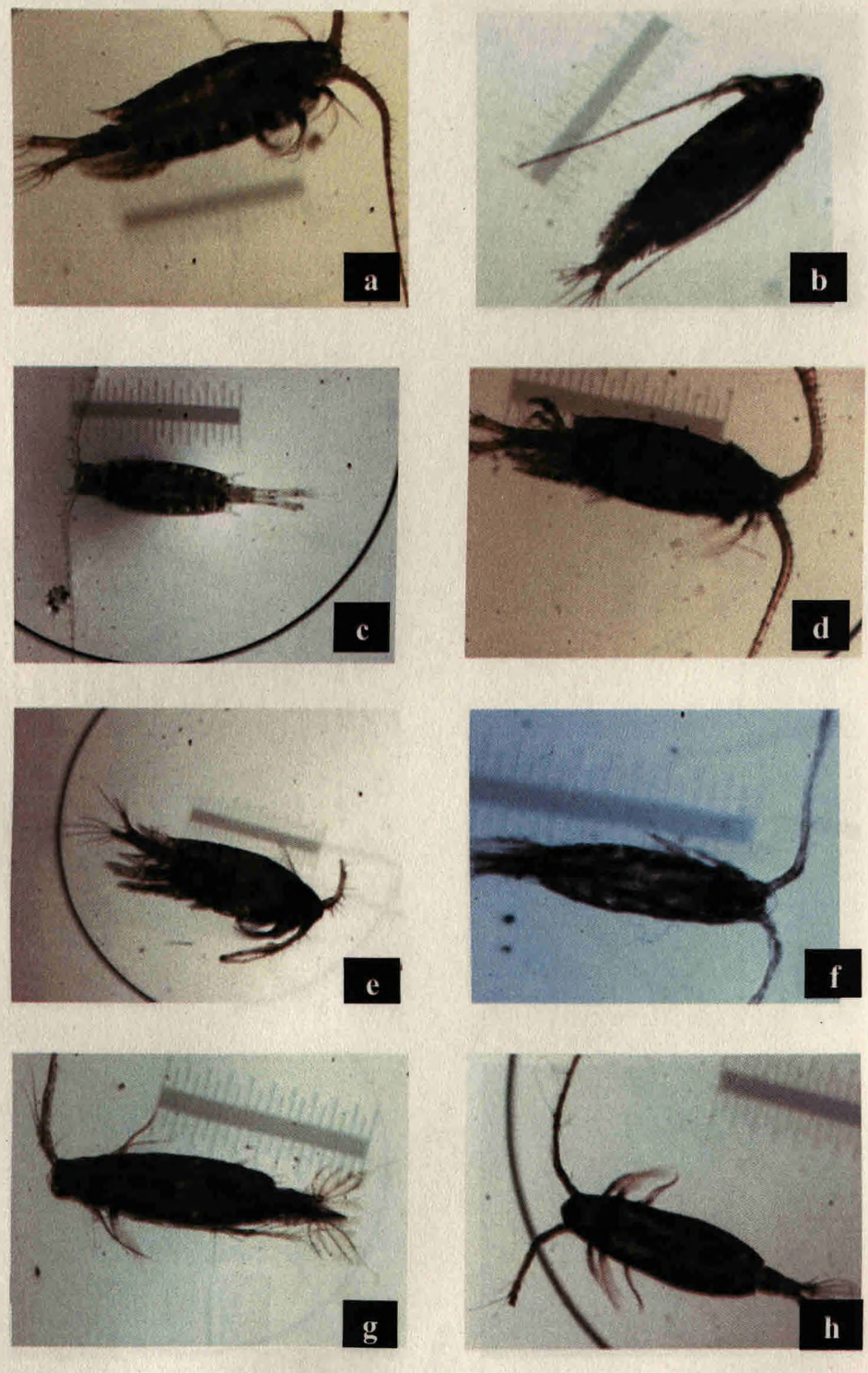


Plate - VII

Plate - VIII



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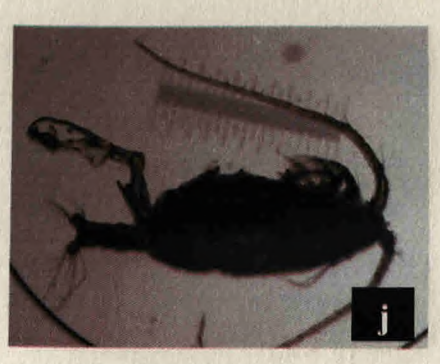
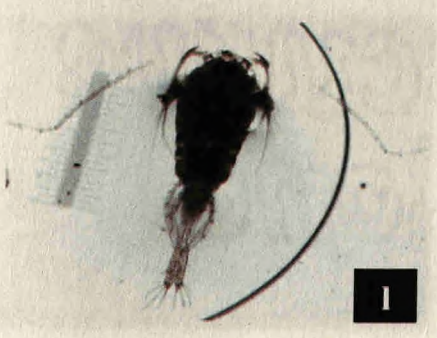
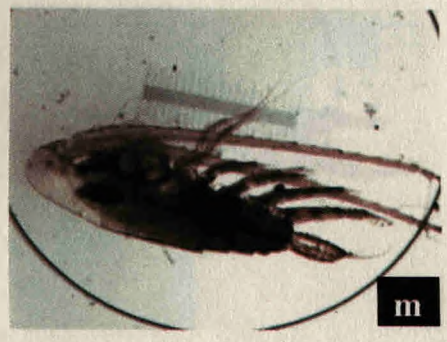






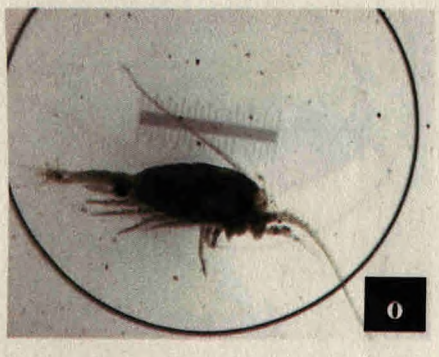
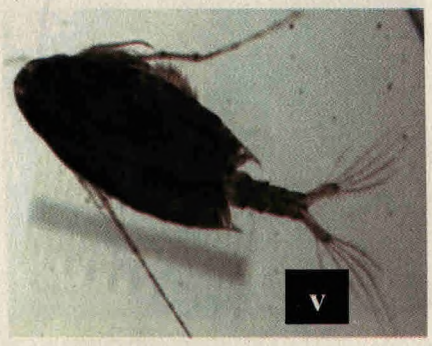


Plate - X



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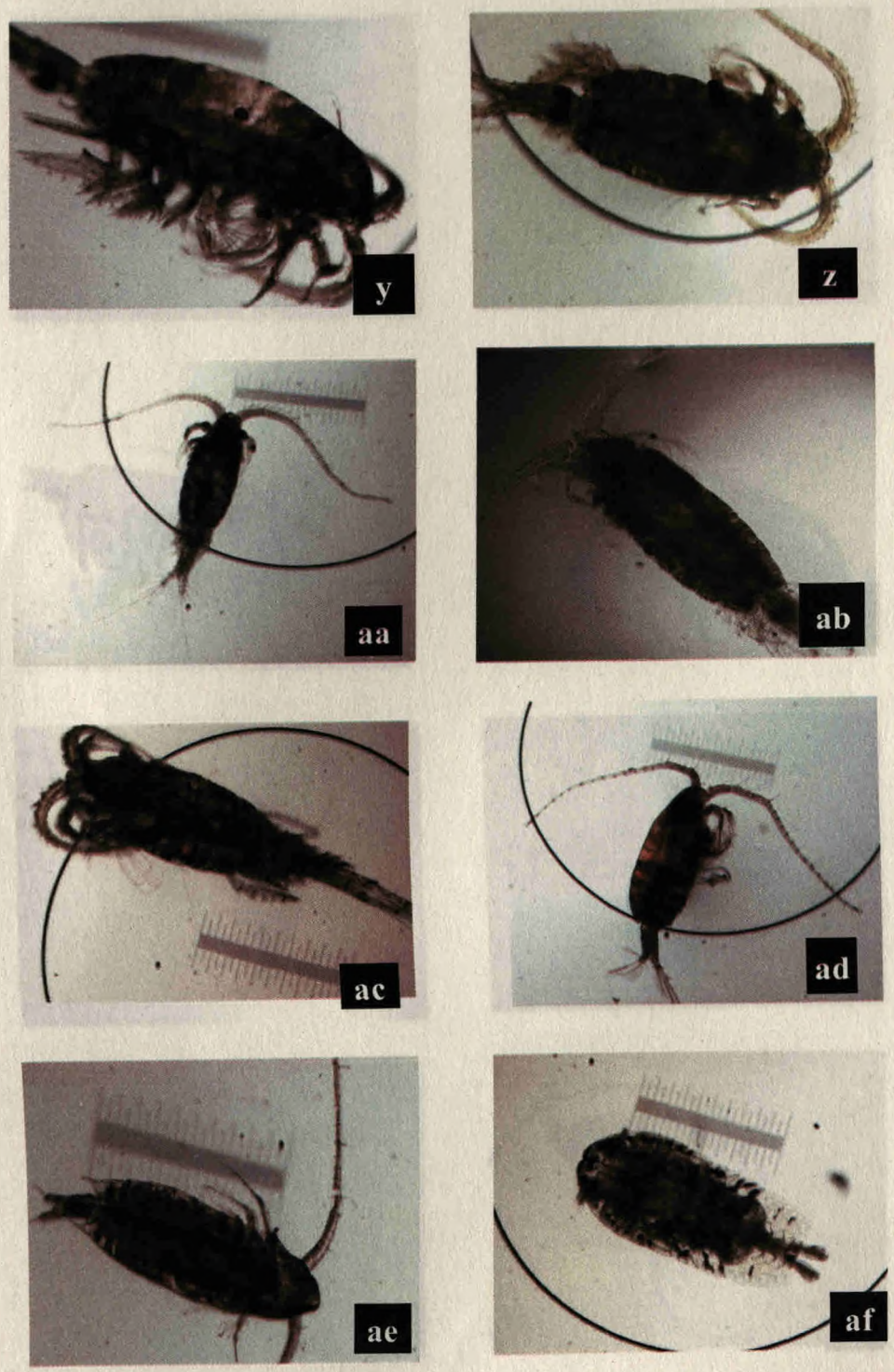

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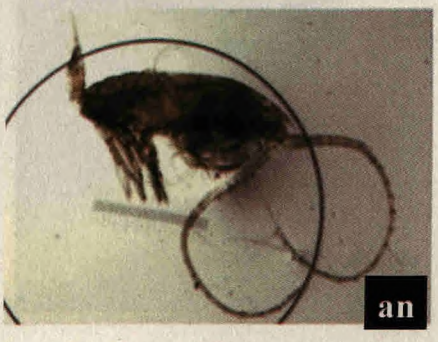
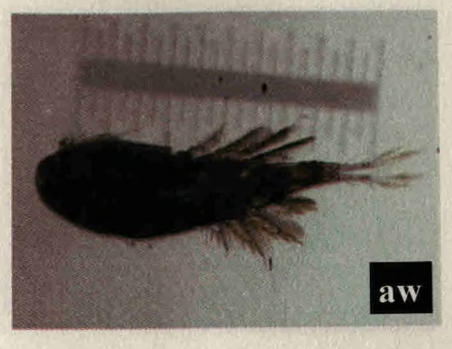
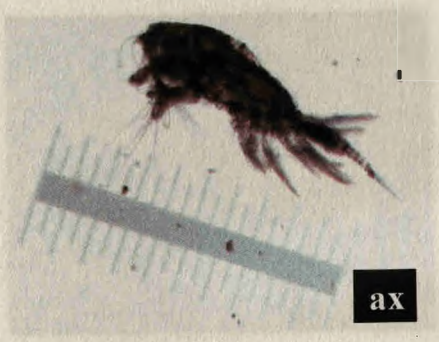
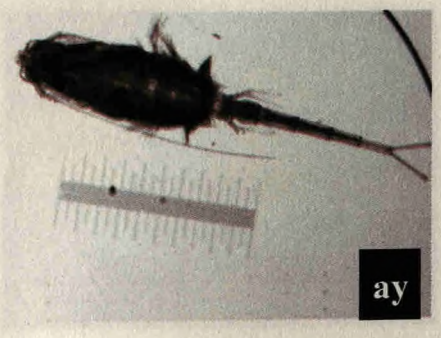


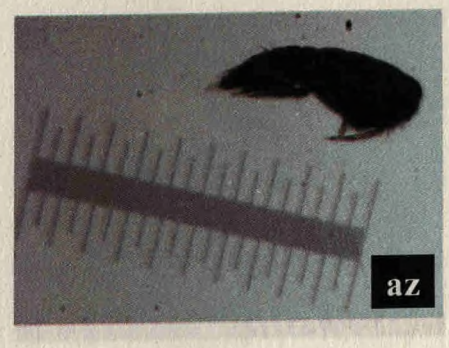
Plate - XIII

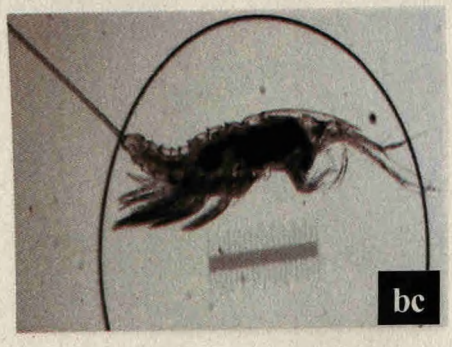
Plate - XIV











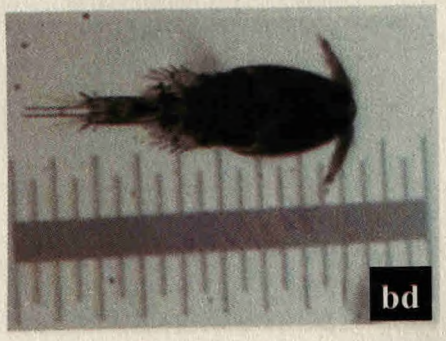


Plate - XV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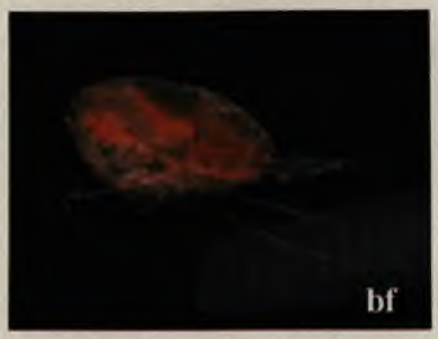
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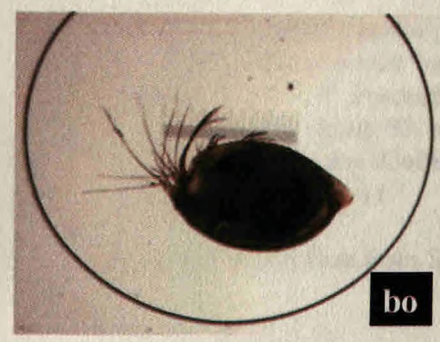
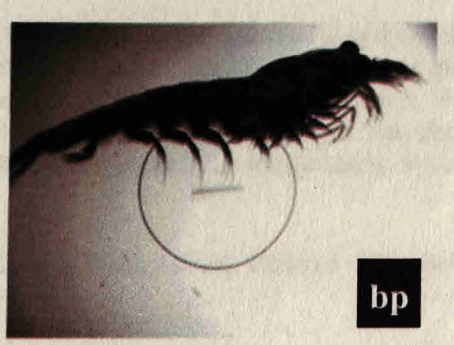
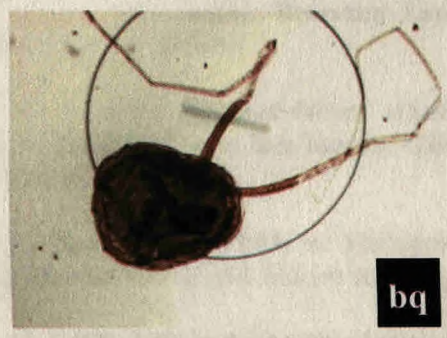
Plate - XVI







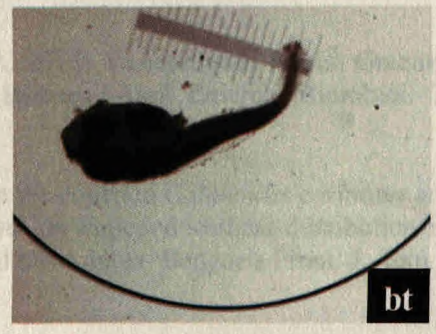


Plate - XVII

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Manuscript I:

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Response of phytoplankton to nutrient enrichment with high growth rates in a tropical monsoonal estuary - Zuari estuary, India

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Present study is about the impact of macronutrient enrichment on phytoplankton biomass and community structure in a tropical monsoonal estuary. *In situ* experiments carried out during the pre-monsoon period (February-March 2006), showed that the response time of phytoplankton to nutrient enhancement was 24-32 h. Phytoplankton biomass increased sizably, indicating nitrate and silicate limitation for phytoplankton growth. An increase in 23µg chl *a* l^{-1} resulted in an uptake of 10µM nitrate, 0.6µM phosphate and 17µM silicate. Phytoplankton showed high growth rates with an average value of 1.36µg chl l^{-1} d^{-1}.This phytoplankton community was largely dominated by diatoms (>96%), particularly chain forming species. Relative preference index (RPI) value for nitrate was >1, suggesting that, irrespective of the ambient ammonium concentration, estuarine autotrophs preferred nitrate. Few species like *Skeletonema costatum and Thalassionema nitzschioides* exhibited the ability to withstand hypoxic condition.

[**Keywords:** Zuari estuary, Premonsoon, Nutrient uptake, Phytoplankton, Hypoxic]

Introduction

Phytoplanktons are responsible for nearly half of global primary production[1]. Diatoms, dinoflagellates, cyanobacteria and coccolithophores are among the most important groups of phytoplankton in the world ocean. A substantial proportion of the coasts include highly productive estuaries[2]. These estuaries, besides supporting a wide variety of animals and plants, act as an important linkage and buffer zone between the ocean and land. They are also sites of high rates of production of organic matter, which not only sustain a secondary food chain internally, but also influence biological productivity of adjacent coastal water in turn sustaining fisheries[2,3]. The extent to which estuaries exchange dissolved and particulate nutrients with adjacent marine ecosystems depends upon several factors, including geomorphology, tidal regime, climate and fresh water inputs. Light and nutrients are the primary factors regulating phytoplankton growth[4,5] followed by temperature and salinity[6]. Major (macro) nutrients essential for plant growth are nitrogen, phosphorous and silicon[7]. Phytoplankton preference for reduced N compounds, primarily as ammonium and urea, is an almost universal phenomenon in marine systems, including estuaries[8,9,10]. Nutrient availability in an estuary is strongly influenced by freshwater flow (river runoff and ground water inputs), atmospheric deposition and exchange with the open ocean. Fixation of dinitrogen is yet another phenomenon of ecological significance known to naturally fertilize tropical waters[11]. In stratified waters, phytoplankton production is primarily enhanced by nitrate supply i.e. new production[12], to the euphotic zone during the active stages of upwelling[13] but, as this supply decreases during the relaxation of upwelling, production is mainly supported by regenerated forms of nitrogen such as ammonium and urea[14,15]. Therefore, phytoplankton must be able to adapt to the changing physical and chemical conditions in these areas[16,17]. This is achieved by storing nitrogen (N) compounds in intracellular pools during periods of N excess, when luxury uptake exceeds growth rates, allowing for the continuation of growth after depletion of external nutrients[18].

In many coastal waters, increasing eutrophication, due to human activities has greatly perturbed the phytoplankton community. With an overwhelming

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majority of the human population living in the coastal zone and with runoff from the continents funneling through estuaries and continental margins, coastal systems are among the most heavily anthropologically impacted ecosystems on the globe. The consequences of eutrophication can only be minimized by identifying the specific nutrient that is limiting to algal growth and primary production. In case of fresh water systems, it is phosphorus[19], whilst in marine system it is generally nitrogen[20]. However, a seasonal shift from phosphorus to nitrogen limitation is observed in coastal transition areas, such as estuaries[21] while limitation in bio available silica has been reported from the subtropical estuary of Taiwan[22].

West coast of India is one of the regions that experiences upwelling bringing low oxygen[23,24], nutrient rich waters eg. NO_3^- enhanced upto 12μM, enters into this estuary[25,26]. In the present study, this effect is simulated by artificially enriching the near mouth estuarine water with inorganic nutrients, in order to understand the dynamics of algal nutrient uptake and its growth and to determine nutrient limitation, if any.

Materials and Methods

Mandovi-Zuari estuarine system is a well-mixed coastal-plain monsoonal estuary situated between latitudes 15° 25' to 15° 31' N and longitudes 73° 45' to 73° 59' E in Goa, along the west coast of India (Fig. 1). Before the incubation experiment, ambient water samples were collected and analyzed for a range of parameters such as pH, temperature, salinity, dissolved oxygen, nutrients, microzooplankton and phytoplankton counts. All samples collected were processed following the JGOFS protocol[29].

Estimation of phytoplankton biomass (chl *a*) was done by flurometer (Turners Design 10AU). For

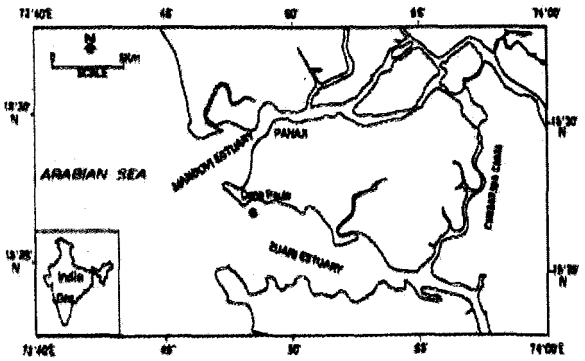

Fig. 1—Map showing study location (solid circle), near the mouth of the Zuari estuary.

qualitative studies of phytoplankton, water samples were fixed with 2% acid Lugol's iodine (1% w/v) and preserved in 3% buffered formalin. The sample was then allowed to settle. Abundance and composition was determined using a Sedgwick rafter plankton counting chamber by means of an inverted microscope (magnification 100-400X). Generic and species identification was done according to taxonomic key[30]. While microzooplankton samples were preserved in 2% acid Lugol's solution, Strontium sulphate solution (2mg l^{-1}) and 1% hexamine buffered formaldehyde and analyzed upto group level under an inverted microscope with phase contrast optics following[31] at 200-400X magnification.

Nutrient samples were collected through BD plastic syringes and immediately stored at -20 °C until analysis. After defrosting, the water samples were analyzed for NO_3^-, NO_2^-, NH_4^+, PO_4^{-3} and SiO_4^{-4}, using a SKALAR segmented flow autoanalyser. Samples for oxygen were collected in gas tight Hamilton glass syringes and were fixed immediately by adding Winkler A and Winkler B solution. The precipitate was subsequently dissolved by acidification and the absorbance of developed color was measured at 456 nm[32] using a Shimadzu UV–visible spectrophotometer.

Two nutrient enrichment experiments were conducted in February and March 2006. Nalgene bottles (25.5 l capacity) were modified for this purpose by drilling two holes through the cap of the bottle through which nylon tubes were inserted, one reaching the bottom of the bottle to draw the sample, and the other to replace the volume of the water removed with helium. The outer ends of each tube were fitted with a three way valve and the entire system was ensured to be airtight (Fig. 2).

Water samples from 1m depth were drawn using a Niskin sampler and screened slowly and

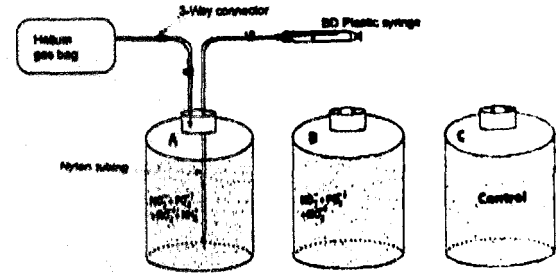

Fig. 2—A schematic diagram of the experimental-1 setup. Bottle (A): enriched with NO_3^-, PO_4^{-3}, SiO_4^{-4}, and $NH4^+$; Bottle (B): enriched with NO_3^-, PO_4^{-3} and SiO_4^{-4} and Bottle (C): acts as a control without any additional nutrients.

carefully through a 200μM nylon mesh to exclude macrograzers, without creating much turbulence to avoid damage to delicate organisms such as ciliates. The first bottle (bottle-A) was enriched with NO_3^-, PO_4^{3-}, SiO_4^{-4} and NH_4^+; the second bottle (bottle-B) was spiked with NO_3^-, PO_4^{-3}, and SiO_4^{-4} and the third bottle (bottle-C) was used as a control without any addition of nutrients. Ambient concentrations of nutrients (μM) before incubation were NO_3^- = 0.51, NO_2^- = 0.09, PO_4^{-3}= 0.57, SiO_4^{-4} = 10.92 and NH_4^+ = 2.28. The concentrations were enhanced to 11.7μM NO_3^- , 0.93μM PO_4^{3-}, 18.9μM SiO_4^{4-} and 4.5μM NH_4^+. Bottles were deployed at 1m depth by hanging from a moored floating raft. Depth of incubation was chosen based on previous measurement (T. Suresh, personal communication) showing on an average 330.18 Mmol photons $m^{-2}s^{-1}$, which is equivalent to the near surface PAR value of SW monsoon period (391.34 Mmol photons $m^{-2}s^{-1}$). Amount of light available at the incubation depth is ca. 50-60%. The first sampling was done after 16h of incubation and subsequently after every 24h. Samples from each bottle were drawn almost at the same time using plastic BD syringes. Care was taken to ensure that the bottles were uniformLy mixed prior to sampling. Volume of water drawn was replaced simultaneously with helium from air tight gas bags. The incubation lasted for ~11 days.

The second experiment (experiment-2) was conducted in March, wherein bottle-A was enriched with NO_3^-, PO_4^{-3}, and SiO_4^{-4} and the second bottle, bottle-B, was enriched with nutrients similar to bottle-A, but deoxygenated by purging helium gas. This bottle was initially maintained at hypoxic level (<2 mL O_2 l^{-1}), while bottle-C served as a control. Physico-chemical characteristics of the estuary were quite similar to those in February and the ambient nutrient concentration of NO_3^-, NO_2^- , NH_4^+, PO_4^{-3}, and SiO_4^{-4} were 0.35, 0.12, 0.46, 0.67 and 8.41μM respectively. Unlike experiment-1, samples were collected at much closer time intervals i.e. every 4 hours during the first 32h; and subsequently at 24 hourly interval for a period of 5 days.

Results

Experiment –1 (2-13 February 2006)

Nutrient uptake

This experiment was initiated during high tide (2.07 m, 13:40 h), and the bottles were incubated *in situ* at 16:00 h. The first sampling (T1) was done after 16h of incubation. Due to the delayed incubation on the first day, only 3 hours sunlight was available for photosynthesis, hence there was no significant decrease in nutrient concentrations. Bottles A and B showed a drastic drop of NO_3^-, PO_4^{-3} and SiO_4^{-4} levels between 16 and 40h of incubation. The decreases in nutrient concentrations coincided with a sharp increase in chlorophyll *a* concentration. In the control bottle (bottle-C) the chlorophyll concentration decreased with time (Fig. 3). The phytoplankton biomass which showed an increase to 23μg chl *a* l^{-1} resulted in the utilization of 10μM nitrate, 17μM silicate, and 2.2μM ammonium in bottle-A. Similarly, in bottle-B chlorophyll increased to 22.5μg chl *a* l^{-1} with concomitant utilization of 8μM NO_3^-, 0.6μM PO_4^{-3}, 15μM SiO_4^{-4} and 0.8μM NH_4^+, after 16h of incubation. After 40h of incubation 21μg chl *a* l^{-1} resulted in the utilization of 12μM, 0.6μM, 19μM and 2.4μM of NO_3^-, PO_4^{-3}, SiO_4^{-4}, and NH_4^+, respectively, from the initial concentrations in bottle- A. While, in bottle-B an increase of 20μg chl *a* l^{-1} resulted in utilization of 11μM, 0.34μM, 19μM and 1μM of NO_3^-, PO_4^{-3}, SiO_4^{-4} and NH_4^+ , respectively.

Thus, after 16h of incubation (up to 64h) concentration of nutrients in bottle-A decreased by 84-98% for NO_3^- , 58-66% for PO_4^{-3} , 88-100% for SiO_4^{4-} and 50-54% for NH_4^+ and in bottle- B, NO_3^- decreased by 65-94% , 34-61% for PO_4^{3-} , 77-100% for SiO_4^{-4} and 30-45% for NH_4^+ of the original values while chlorophyll *a* increased by a factor of three. It clearly indicates that NO_3^- and SiO_4^{-4} were nearly exhausted after 40h of incubation (Fig. 3). Every 1 μM decrease in NO_3^- resulted in 2.3-2.9 (avg. 2.6) μg L^{-1} chl *a* gain in phytoplankton biomass. This gain was particularly seen between 16 and 40h of incubation. Though, NH_4^+ was also taken up by phytoplankton along with NO_3^- (and NO_2^-), the uptake rate was comparatively much lower. However, after 3.5 days when NO_3^- had been depleted, NH_4^+ was still available in the medium that perhaps supported secondary chlorophyll peak in bottle-A with 17μg chl *a* L^{-1} and in bottle-B with 8μg chl *a* l^{-1} coinciding with the decline in NH_4^+ concentration after 136 and 88h of incubation in bottle-A and bottle-B, respectively.

The enclosed water remained well oxygenated (>4mL O_2 l^{-1}) throughout the experiment. Hence, the decrease in NO_3^- should be entirely due to the uptake by phytoplankton. Significantly, NO_3^- was preferred over NH_4^+. Difference in NO_3^- uptake pattern in bottle

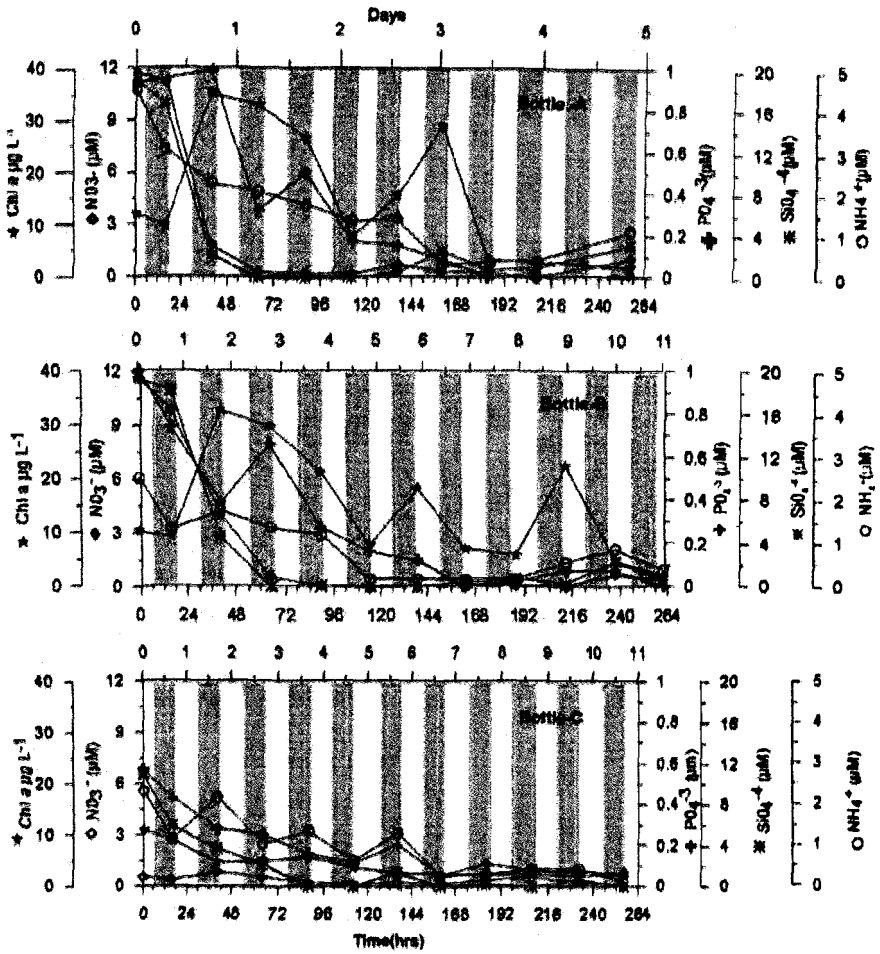

Fig. 3—Comparative variations in chl *a* and nutrient concentrations with time of incubation in February (experiment-1). Bottle (A): NO_3^-, PO_4^{-3}, SiO_4^{-4}, and $NH4^+$; Bottle (B): NO_3^-, PO_4^{-3} and SiO_4^{-4}, and Bottle (C) without any additional nutrients (control). Shaded portions represent dark periods.

A and B was found to be insignificant (ANOVA, p= 0.9) indicating that the uptake pattern of nutrients and biomass growth were similar in both the bottles. Variations between the data sets for all the parameters between the two bottles were insignificant (p>0.05) indicating bottle-A and bottle-B behaved almost as duplicates. Net growth rates (μ) from changes in the chlorophyll biomass[33] were calculated to be 1.24 and 1.23μg chl L^{-1} d^{-1} in bottle A and B, respectively. This closeness of μ in both bottles indicates that presence of NH_4^+ did not cause any significant change in algal biomass as long as NO_3^- was available, and at the same time NH_4^+ did not suppress uptake of NO_3^- in bottle-A. While bottle-C showed -0.67μg chl L^{-1} d^{-1} due to high grazing pressure exerted by the microzooplankton, tintinnids in particular and lack of nutrients to support further build up of phytoplankton biomass (Fig. 8).

Phytoplankton composition

It was observed that chlorophyll *a* in both the treated bottles responded in similar pattern. Likewise, the phytoplankton abundance also showed similar trends except at 40h when there was a relative increase in abundance in bottle-B as compared to bottle-A (Fig. 4a). This high value in bottle-B was due to the outburst of *Thalassionema nitzschioides*, *Thalassiosira subtilis*, *Asterionella japonica* and *Chaetoceros curvisetus* (Fig. 4b). The phytoplankton assemblage was composed of 42 species (diatoms-35; dinoflagellates-6, and silicoflagellate-1) in the experimental bottles. Cell density varied from 1.4×10^5 to 3.0×10^6 cells l^{-1} in bottle-A and from 1.1×10^5 to 5.1×10^6 cells l^{-1} in bottle-B. The control bottle showed a range from 1.2×10^5 to 8.3×10^5 cells l^{-1} in 40h. The diatoms accounted for 99% of the total phytoplankton community. The dominant species

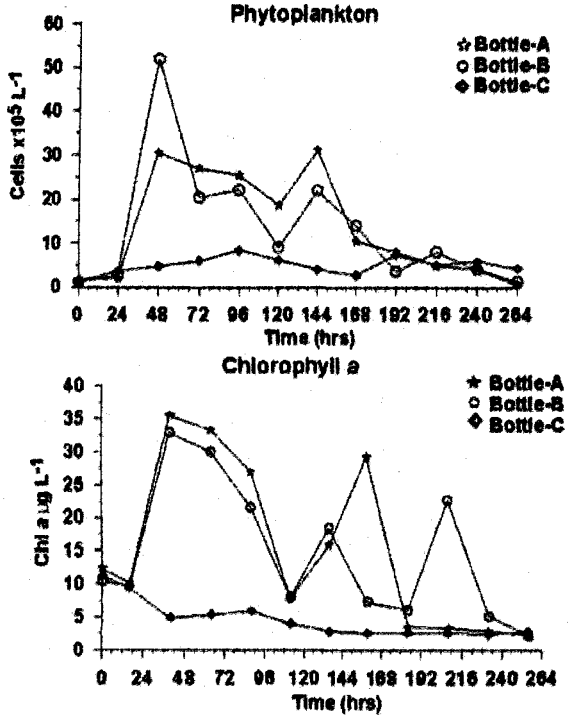

Fig. 4(a)—Variations in phytoplankton abundance and biomass, in Bottle (A) and Bottle (B) during February (experiment-1).

were *Thalassionema nitzschioides, Thalassiosira subtilis, Asterionella japonica, Chaetoceros curvisetus, Melosira* sp, *Skeletonema costatum, Chaetoceros lorenzianus, Guinardia striata, Guinardia delicatula, Leptocylindrus danicus, Pseudo-nitzschia seriata, Dactylisolen* sp and *Nitzschia closterium.* Among these, *Thalassionema nitzschoides, Asterionella japonica, Pseudo-nitzchia seriata,* and *Nitzschia closterium* are chain-forming pennates while others are centric diatoms. However, dinoflagellates like *Ceratium* sp, *Gymnodinium* sp, *Prorocentrum* spp, and *Protoperidinium* spp were negligible in the total community. *Dictyocha fibula* was the only representative of silicoflagellates.

Experiment –2 (25-30 March 2006)

Nutrient uptake

In the first experiment, the bottles were exposed to sunlight only for ~3 h after their deployment. The reason for low uptake of nutrients during the first 16h whether it was due to lack of photosynthesis at night or a time lag in phytoplankton response could not be ascertained. Therefore, the experiment was repeated to resolve this issue. In addition, one bottle,

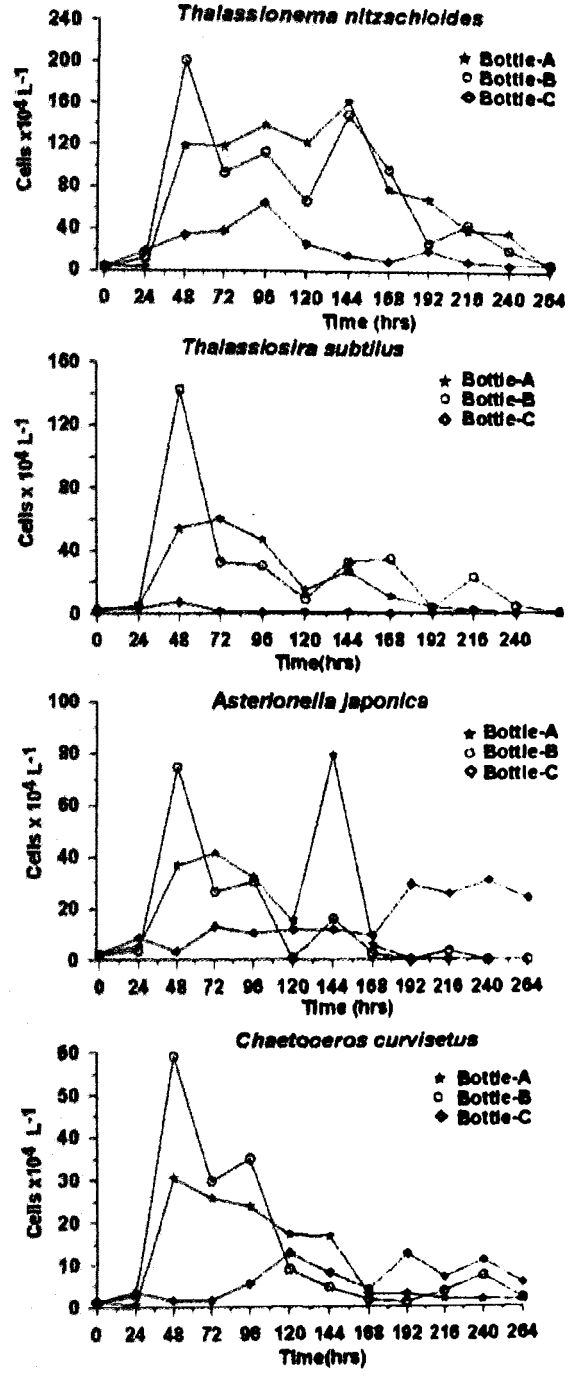

Fig. 4(b)—Variations in abundance of some diatom species viz. *Thalassionema nitzschioides, Asterionella japonica, Chaetoceros curvisetus* and *Thalassiosira subtilis* with incubation time in Bottle (A), (B) and (C) during February (experiment-1).

bottle-B was kept of O_2 <2 mL L^{-1} (hypoxic) but enriched with nutrients. This was to determine the response of phytoplankton cells when exposed to

deoxygenated waters, as happens naturally during the late phase of southwest monsoon when the study area experiences incursion of upwelled waters[24]. In this experiment, the bottles were exposed to sunlight for the whole day light period (11 h). All the bottles were deployed early in the morning and sampling was done at much shorter time intervals (every 4 hours) within the first 32h to closely monitor nutrient uptake pattern and thereafter at 24 h intervals.

During this experiment, all bottles were incubated well before sunrise after enriching with nutrients. Sharp decreases in nutrients (NO_3^- by 9μM; PO_4^{-3} by 0.3μM and SiO_4^{-4} by 12μM) with a concomitant increase of 19 μg l^{-1} chl *a* in the bottle-A at ~32h of incubation was observed (growth rate = 1.4μg chl *a* l^{-1} d^{-1}). As expected, changes in these parameters were negligible in the control, bottle-C (Fig. 5), and the difference between the initial and final nutrient and chlorophyll concentrations were still found to be statistically insignificant (ANOVA, p=0.9).

Bottle-B was gradually subjected to low oxygen hypoxic conditions, attained within 2 hr, by purging with helium gas. Dissolved oxygen was maintained low with <2 mL O_2 l^{-1} but, after 4-8 h of incubation oxygen level increased to ~3 mL O_2 l^{-1} and remained consistent throughout the experiment except at nights. A close coupling between chlorophyll *a* and dissolved oxygen due to photosynthetic activity is shown in Figure 6. The NH_4^+ concentration in bottle-B dropped by 50% while in other bottles it showed only 5% decline, while NO_3^- and SiO_4^{-4} decline was negligible with only 8 and 3% respectively of their initial concentration in bottle- B. On the whole, substantial nutrient decline and chlorophyll buildup (i.e. 5 to

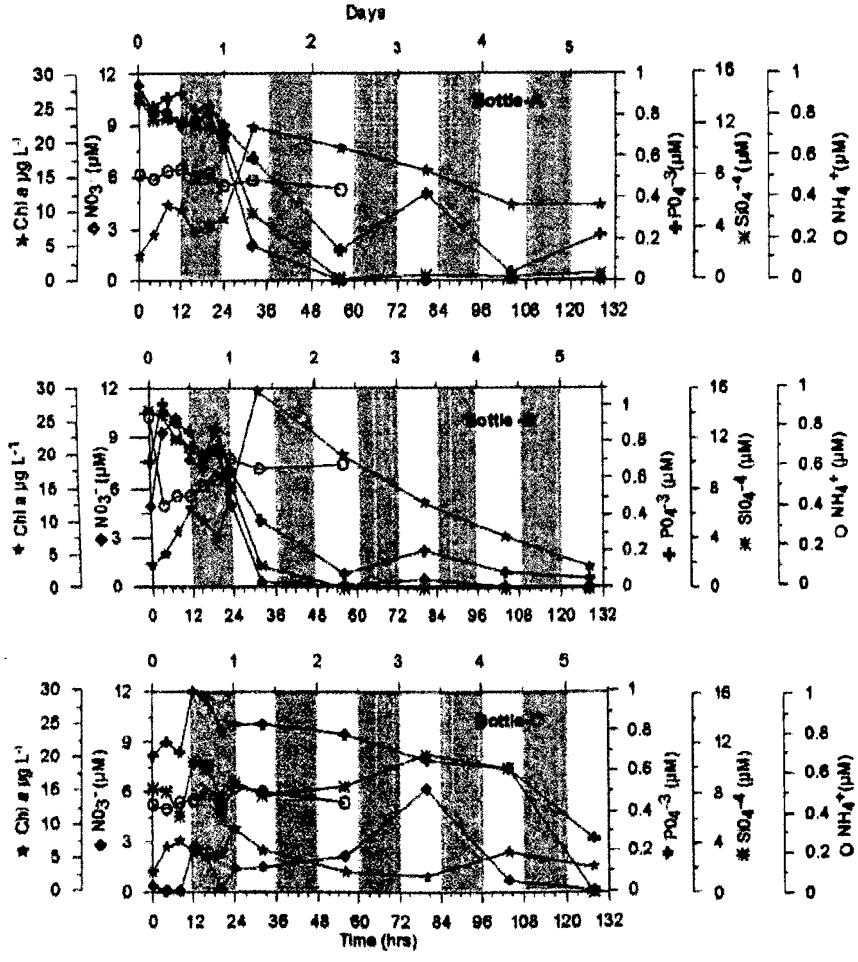

Fig. 5—Comparative variations in chl *a* and nutrient concentrations with time in March (experiment- 2). Bottle (A): with added nutrients NO_3^-, PO_4^{-3}, SiO_4^{-4}; Bottle (B): with nutrients, as in bottle (A), but deoxygenated (< 2 mL O_2L^{-1}) and Bottle (C): without any additional nutrients as control.

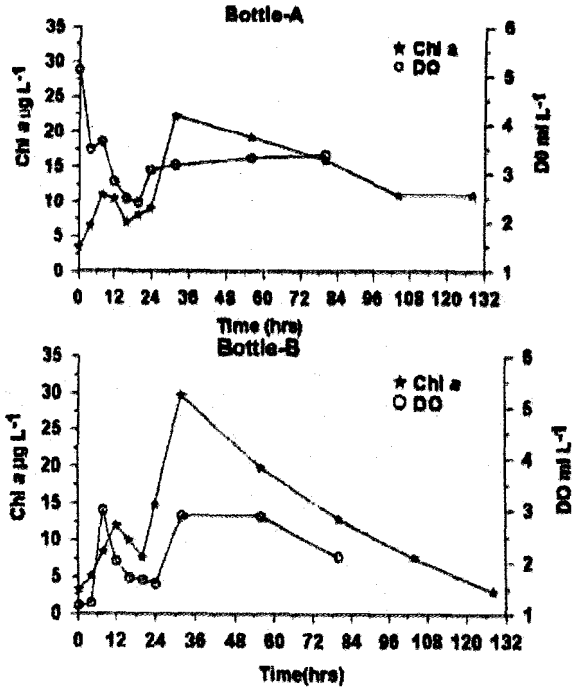

Fig. 6—Co-variations in chlorophyll *a* and dissolved oxygen in Bottle (A) and (B) in March (experiment- 2).

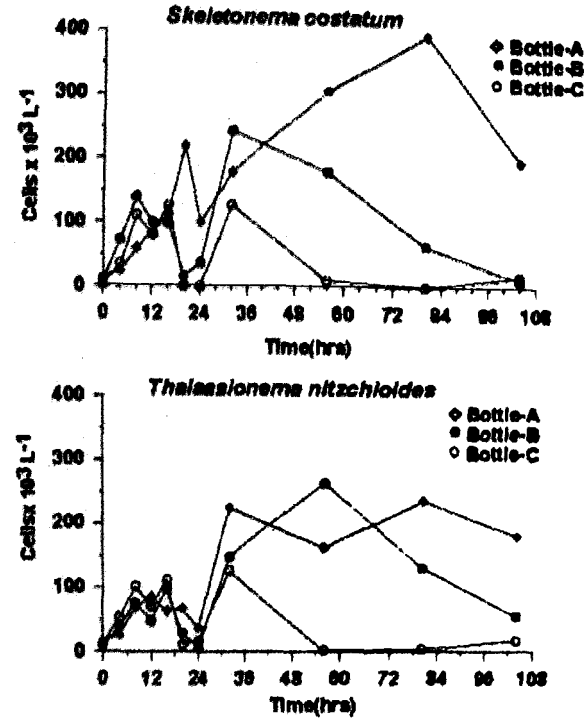

Fig. 7(a)—Variations in cell abundance of some diatom species viz. *Skeletonema costatum* and *Thalassionema nitzchioides* with incubation time in Bottle A, B and C during March (experiment-2).

30µg chl *a* l^{-1}) with rapid uptake occurred only after 20h of incubation (earlier by 4h) in deoxygenated bottle as compared to other bottles. The growth rate was found to be comparatively higher with 1.6 (µg chl *a* L^{-1} d^{-1}) in bottle- B than in control bottle (0.6).

Phytoplankton composition

The phytoplankton composition was similar to experiment-1 and composed of 45 species (diatoms-28; dinoflagellates-7, silicoflagellate-1 and blue green algae-1). Cell density in bottle- A varied from 5.7×10^4 to 7.7×10^5 cells l^{-1}. The control bottle showed a range from 3.5×10^4 to 0.8×10^5 and in bottle-B counts ranged from 7.5×10^4 to 8.6×10^5 cells l^{-1} in 32h. In general, diatom accounted for 98% of the total algae community. These high values in bottles-A and B were again due to the dominance of fast growing chain forming diatoms viz. *Skeletonema costatum, Thalassionema nitzschioides, Chaetoceros curvisetus, Chaetoceros lorenzianus, Leptocylindrus danicus and Guinardia striata.* Further, some species showed gradual increase from 4-12 h which coincided with the rise in chlorophyll *a* and oxygen production particularly, *Skeletonema costatum* and *Thalassionema nitzschioides* in bottle-B (Fig. 7a). Conversely, some forms viz. *Thalassiosira subtilis, Ditylum brightwellii, Melosira* sp *and Rhizosolenia crassispina* were low in abundance in bottle-B as compared to the control bottle and showed increase only after 24 hrs. (Fig. 7b).

In general, the phytoplankton composition did not vary much among the bottles. However, numerically cells were higher by 70% in bottles-A and B compared to the control bottle. Similar to the first experiment, maxima in phytoplankton density, was observed at 32h of incubation, clearly coinciding with high chl *a*, which decreased gradually with time and was comparable to control bottle after 182h. Diatoms always remained the dominant group comprising >96% of the algal community. Dinoflagellates, silicoflagellates and diazotroph *Trichodesmium erythraeum* collectively formed <5 % of the total phytoplankton community.

Discussion

Results of incubation experiments provide valuable insights into the response of natural phytoplankton assemblages to nutrient enrichment. Previous results

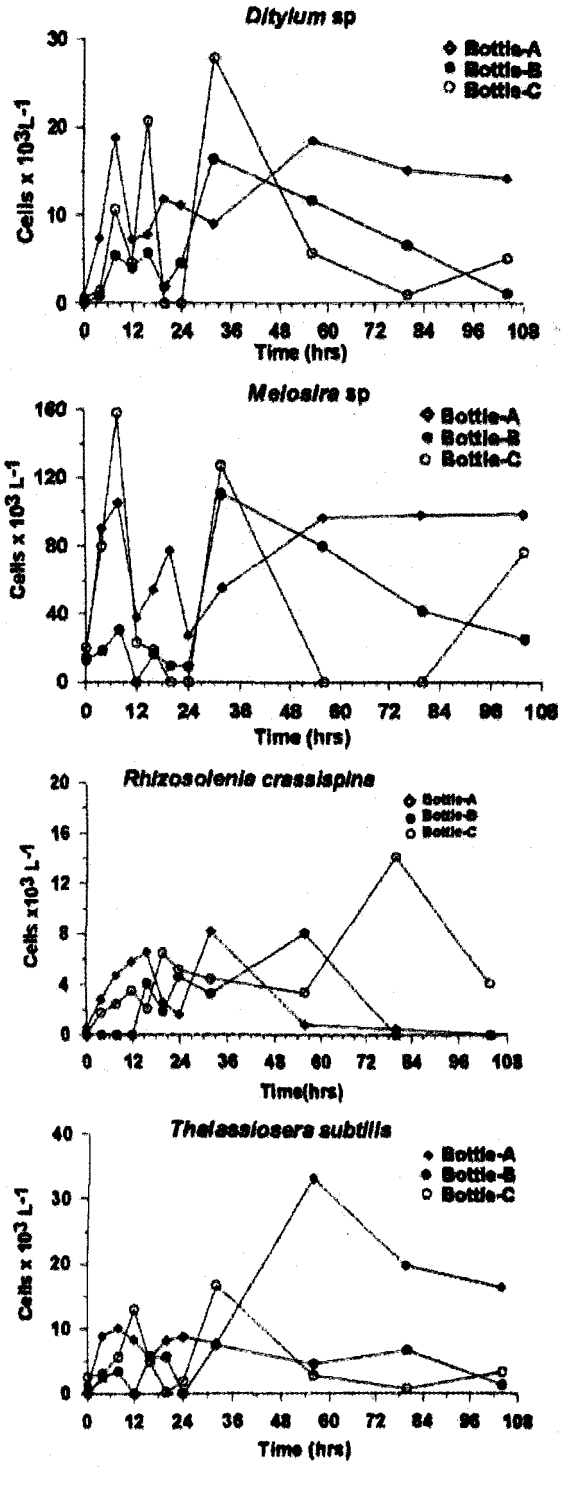

Fig. 7(b)—Variations in cell abundance of some diatom species viz. *Ditylum* sp; *Melosira* sp; *Rhizosolenia* sp; *Thalassiosira* sp with incubation time in Bottle (A), (B) and (C) during March (experiment-2).

from enclosure experiments have been useful to describe processes operating in natural conditions[34]. Such experimental approach has revealed how nutrient limitation may affect algal growth rate and net biomass accumulation. Nutrients are largely assimilated by phytoplankton during the day for photosynthesis. From the above two experiments, it is apparent that the response of estuarine phytoplankton to nutrient enrichment is almost immediate. An increase of 19-26 (avg. 23) μg chl *a* l^{-1} resulted in loss of 8-10 (avg. 9) μM NO_3^-, 0.3-0.6 (avg. 0.45) μM PO_4^{-3} and 9-17 (avg. 13) μM SiO_4^{-4}. The observed rapid phytoplankton uptake within 24h of nutrient enrichment in the study region may be true for other tropical estuaries. There does, however, appear a period of few hours when the uptake is relatively slow, as observed particularly during the experiment-2 conducted in March. The lower initial uptake rate may be due to physiological adaptation of phytoplankton to enrichment.

Studies with unialgal laboratory cultures and artificially enriched coastal seawater have shown that marine phytoplankton prefer NH_4^+ over NO_3^- as a nitrogen source[35]. Several other authors[36,37] have also reported the inhibitory effect of NH_4^+ on NO_3^- which severely reduces the rate of NO_3^- uptake. Further, some studies found a threshold ammonium concentration of 1 μM, above which NO_3^- uptake is largely inhibited despite high concentration of ambient NO_3^-[18,38]. Similar conclusion was drawn based on the theoretical consideration of the relative energy requirement for the utilization of NO_3^- and NH_4^+ [39,40]. Some reports have shown simultaneous uptake of NO_3^- and NH_4^+ [41,42]. But, unlike others, a preference for NO_3^- over NH_4^+ was also observed[43]. In nitrogen replete cultures, studies have shown enhanced metabolism of NO_3^- with increase in irradiance[44]. Other studies have found that, the half saturation constant for nitrate uptake was related to temperature[45] where higher temperatures enhance NO_3^- utilization[46]. In our study, experiment-1, NH_4^+ concentration was >2μM, but no inhibition was observed in the bottles A and B. Instead, we found that NO_3^- was taken up before NH_4^+ and the uptake pattern of nutrients and biomass growth appeared to be similar in bottle-A and bottle-B. No significant difference was found between these two data sets ($p>0.05$). These results suggest that the phytoplankton community of monsoonal estuary preferred NO_3^- over NH_4^+ and possibly have a higher NH_4^+ threshold.

Though in the experiments NH_4^+ did not seem to contribute directly to the growth of biomass, as NO_3^- was the preferred species, still its conversion to NO_3^- via nitrification, can indirectly meet the N requirement of phytoplankton. Further, NH_4^+ can also be made available through re-mineralization, as seen after 5 days of incubation in experiment-1 which boosted the secondary chlorophyll peak. It also leads to speculation that NH_4^+ is taken up significantly when ambient NO_3^- concentration is low. This speculation arises from the fact that although NO_3^- remains low (<1µM) as compared to PO_4^{-3} (>0.5µM) or SiO_4^{-4} (>8µM) in the non monsoon seasons, maximum primary production occurs during the premonsoon (March-April) and post monsoon (Oct-Nov) periods [47,48] being supported by regenerated nutrients such as NH_4^+. However, the DIN in the Zuari estuary was observed to remain low unless there are some episodic inputs of nutrients. This was clearly seen through higher uptake rates in the experimental bottles. Molar ratios of nutrients in the water at time of experiment-1, (DIN/DIP= 5.05; and DIN/Si= 0.26) and in experiment-2 (DIN/DIP= 1.39; and DIN/Si= 0.11) was much lower than the Redfield values suggesting N limitation in this region during the pre-monsoon season. The nutrient uptake of nitrate, phosphate and silicate by the phytoplankton community was taken up close to the Redfield ratio in February and March (Table 1).

However higher DIN: DIP (>16) in March indicates that nitrogen is assimilated at slightly faster rate (Table 2) possibly due to enhanced solar radiation. NH_4^+ has been found to be present at a concentration ~4µM in post monsoon season (Oct-May), which may enter the system from nearby mangrove swamp, sewage discharge or re-suspension of sediment and benthic regeneration[49]. Further, nitrogen fixation by *Trichodesmium* that occurs every year starting from late January to May, makes a substantial contribution to the total nutrient budget in the region[11]. Following the decay of this bloom, large amount of NH_4^+ (up to 3.3 µM) is released into the medium[11], which leads to proliferation and succession of other planktonic organisms[50]. *In situ* measurements of benthic fluxes[49] have shown that the estuarine sediment is a sink for NO_3^- whereas NH_4^+ remains the dominant N form that is released from sediments in a significant quantity in premonsoon months. Only during the monsoon season (June-Sept) the estuarine waters get enriched with NO_3^- (~8µM) along with PO_4^{-3} (2.5µM) and SiO_4^{-4} (>60µM)[51] but even then, NO_3^- remains unutilized, because of cloud cover and turbidity which results in low algal productivity (61.7mmol C m^{-2} d^{-1})[48]. Thus, NH_4^+, probably supports the estuarine productivity in non monsoon period.

Large sized phytoplankton cells are known to preferentially assimilate NO_3^- over NH_4^+ [52,53]. Studies on nitrogen uptake by size-fractionated plankton showed that the NO_3^- was utilized by net plankton (20-200µM size) and NH_4^+ by nanoplankton (0.8-20µM size)[46,54] .This shifts in N-uptake from NO_3 to NH_4 was also seen during the present study (see Fig. 3), possibly due to the community shift to picoplankton. Hence, most of the dominant taxa were the diatoms (>10µM size) that preferred NO_3^- while other smaller forms must have preferred NH_4^+which is evident from the secondary chlorophyll peak. Several authors[9,55] have used relative preference index, RPI to study the preferential N uptake. A

Table 2—Comparative uptake ratio of DIN. DIP and Si by estuarine phytoplankton

	Experiment-1 (February)		Experiment-2 (March)	
Bottle	DIN/DIP	DIN/Si	DIN/DIP	DIN/Si
A	15.85	0.99	18.78	1.23
B	13.07	0.51	16.20	0.85
C	12.36	0.38	18.29	1.11

Table 1—Comparative uptake of nitrate and ammonium by estuarine phytoplankton. Exp.1-Bottle (A) enriched with NO_3^- and NH_4^+ ; Bottle (B) with nitrate and Bottle (C) as control . Exp.2- Bottle (A) and (B) enriched with only NO_3^-

	Experiment-1(February)					Experiment-2 (March)				
N-Nutrient	Bottle	% Nutrient	% Nutrient utilized	uptake rate (µmol $L^{-1}h^{-1}$)	RPI	Bottle	% Nutrient	% Nutrient utilized	Uptake rate (µmol L^{-1} h^{-1})	RPI
NO_3^-	A	71	84	0.39	1.19	A	93	87	0.78	1.04
NH_4^+	A	27	49	0.01	0.19	A	0.04	14	0.003	0.06
NO_3^-	B	81	65	0.18	1.16	B	84.15	93.9	0.377	1.02
NH_4^+	B	17	30	0.01	0.41	B	14.48	30.58	0.008	0.5
NO_3^-	C	17.7	65.33	0.39	1.172	C	37.63	89.3	0.311	1.18
NH_4^+	C	79.1	30.27	0.033	0.036	C	49.46	6.5	0.02	0.3

consistent value of RPI (>1) for NO_3^- in both experiments indicates that NO_3^- is the preferred N substrate for phytoplankton in the estuary irrespective of ambient NH_4^+ concentration (Table 1).

Diatoms have evolved a multitude of morphologies, which serve as protection against grazing or affect sinking[56]. The impact of cell shape and chain forms can be used to predict uptake of nutrients under turbulent environments[57]. Silicate, an essential nutrient for diatoms, was in surplus to support their build up. But high silicate and low nitrate as in this estuary might limit the phytoplankton growth. Therefore, in this experiment, silicon and nitrogen were added in equal proportions as most diatoms incorporate them in their molar ratio of about 1:1 [58]. It is also evident that along with NO_3^-, SiO_4^{-4} is also limiting for diatoms in this monsoonal estuarine system. This was apparent in these experiments where, fast growing dominant diatoms, viz. *Thalassionema nitzschioides, Thalassiosira subtilis, Asterionella japonica, Chaetoceros curvisetus, Chaetoceros lorenzianus, Guinardia striata, Guinardia delicatula, Skeletonema costatum* and *Leptocylindrus danicus* showed momentous increase and accounted for >96% of phytoplankton. However, the subsequent decrease in the chlorophyll after 32h possibly occurs due to grazing pressure exerted by the micro-grazers such as ciliates in particular (Fig. 8), which followed the chlorophyll peaks.

Some phytoplankton species viz., *Melosira* sp., *Rhizosolenia crassispina* and *Ditylum brightwellii*, in bottle-B of experiement-2 were found to be initially low in abundance during hypoxia (Fig. 7b), but showed an increase after 24h indicating that as oxygen level built up they had potential to bloom given the right conditions.While *Thalassiosira* sp remained invariably low throughout the incubation period w.r.t. bottle-A and C (Fig. 7b). The buildup of oxygen in the bottle-B coincided with the increase in chlorophyll, although cell counts were low (Fig. 6). This suggests that, larger forms (>10µm) were under stress, while smaller fractions were perhaps efficient under low oxygen conditions utilizing NH_4^+ , which dropped to 50% of its initial value after 4h. Thereafter the oxygen level was restored through rapid photosynthesis.

Interestingly, the species *Skeletonema costatum* and *Thalassionema nitzschioides* in bottle-B of experiment-2 remained consistent with Bottle-A (Fig. 7a). These species picked up after 4 h of

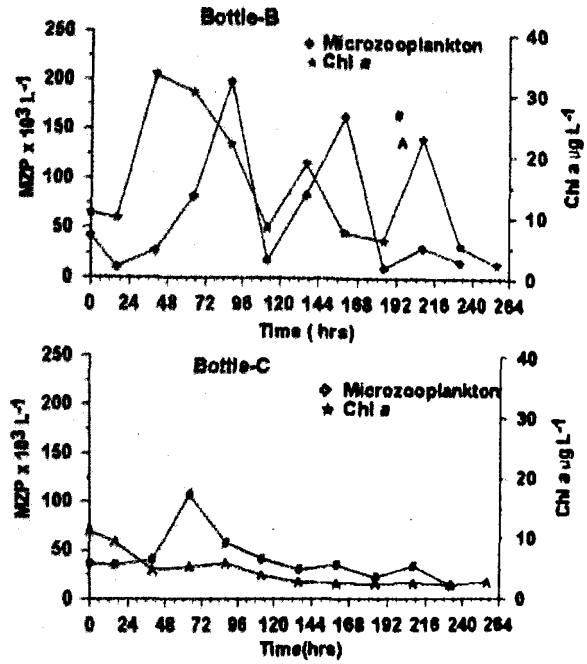

Fig. 8—The grazing effect of microzooplankton on phytoplankton biomass (chl *a*) with time in Bottle (B) and control Bottle (C) in February (experiment-1).

incubation unlike other diatoms signifying that these species have acclimatized under low oxygen conditions and may be thriving in harsh environments such as along the western continental shelf of India, which experiences seasonal oxygen depletion[23]. In support of this view, there is data reported from this coastal region showing few phytoplankton like *Asterionella japonica, Pseudo-nitzschia* sp. *Navicula* spp., *Thalassiothrix* sp., *Thalassionema* spp, *Pleurosigma* sp., and *Skeletomema* sp prevailing during such conditions[59].

Conclusions

Nutrient enrichment experiments were carried out to understand the interaction between the phytoplankton growth and nutrient uptake. Results reveal that the estuarine autotrophs were nitrogen limited during premonsoon period and that the addition of nitrate greatly stimulated the growth leading to biomass accumulation. Presence of considerable amount of NH_4^+ did not show any inhibitory effect on NO_3^- uptake. Rapid uptake of nutrients was observed after a lag phase of 24-32 h and the uptake was significantly dependent on the fast growing diatom taxa that showed high growth rates. *Thalassiosira sp* was one species most sensitive to

low oxygen throughout the incubation period while some species viz. *Melosira sp, Rhizosolenia crassispina, and Ditylum brightwellii* were found to show potential to revive from hypoxic conditions. However, this experiment does not account for an uptake of a regenerated nutrient, which possibly led to an overestimation of N-uptake rates. This warrants more experiment studies to address this issue.

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The relationship between volatile halocarbons and phytoplankton pigments during a *Trichodesmium* bloom in the coastal eastern Arabian Sea

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ABSTRACT

Eukaryotic phytoplankton such as diatoms and prymnesiophytes produce biogenic halocarbons in the ocean that serve as important sources of chlorine and bromine to the atmosphere, but the role of cyanobacteria in halocarbon production is not well established. We studied distributions of chloroform ($CHCl_3$), carbon tetrachloride (CCl_4), methylene bromide (CH_2Br_2) and bromoform ($CHBr_3$) in relation to phytoplankton composition, determined from pigment analysis complemented by microscopic examination, for one month in coastal waters of the eastern Arabian that experienced a *Trichodesmium* bloom that typically occurs during the Spring Intermonsoon season. High concentrations of zeaxanthin (23 $\mu g\ l^{-1}$), alpha beta betacarotene (6 $\mu g\ l^{-1}$) and chlorophyll *a* (67 $\mu g\ l^{-1}$) were found within the bloom whereas the marker pigment concentrations were low outside the bloom. $CHCl_3$ and CCl_4 occurred in relatively high concentrations in surface waters whereas CH_2Br_2 and $CHBr_3$ were restricted to the subsurface layer. Chlorinated halocarbons were positively inter-correlated and with $CHBr_3$. The observed spatial and temporal trends in brominated compounds appear to be related to the abundance of *Trichodesmium* although correlations between concentrations of brominated compounds with various marker pigments were poor and statistically non-significant. The results support the existence of multiple sources and sinks of halogenated compounds, which might obscure the relationship between halocarbons and phytoplankton composition.

1. Introduction

Phytoplankton are known to produce various halocarbon compounds that are environmentally important because of their greenhouse warming potential and ability to destroy ozone in the stratosphere (Lovelock, 1975; Salawitch, 2006; Quack and Wallace, 2003). Oceanic fluxes of biogenic halocarbons contribute significantly to the atmospheric halocarbon budgets (Abrahamsson et al., 2004). The long-lived bromine- and chlorine-containing species are particularly important as they are involved in stratospheric ozone depletion (Chameides and Davis, 1980). Production of these compounds by phytoplankton is species-dependent (Tokarczyk and Moore, 1994). However, in addition to production by phytoplankton (Singh et al., 1983; Tokarczyk and Moore, 1994; Tait and Moore, 1995; Moore et al., 1996; Scarratt and Moore, 1998) and macroalgae (Manley et al., 1992; Ekdahl et al., 1998), anthropogenic activities could also be the source of these compounds in the coastal areas (Mills et al., 1998; Christof et al., 2002) making it difficult to identify their origin. Despite this, co-variation of halogenated compounds and photosynthetic pigments has been taken to support their production by autotrophs (Schall et al., 1997; Yamamoto et al., 2001; Quack et al., 2007; Bravo-Linares and Mudge, 2008). Production of brominated and chlorinated methanes in the open ocean has often been linked to phytoplankton, particularly diatoms (Class and Ballschmiter, 1988; Klick and Abrahamsson, 1992; Baker et al., 2000; Plummer and Edzwald, 2002).

Unlike eukaryotic phytoplankton and macroalgae, information on halocarbon production by cyanobacteria is limited. Karlsson et al. (2008) recently demonstrated the potential of cyanobacteria in the Baltic Sea in the production of halogenated (especially brominated) compounds. *Trichodesmium* is inarguably the best known of all cyanobacterial genera in the ocean. However, little is known about the halocarbon production by this genus, which occurs widely in tropical waters often forming large blooms. These blooms are, however, quite patchy, both temporally and spatially. The patchy distribution is usually connected to the physical characteristics of surface waters (Kononen and Leppänen, 1997). In the Arabian Sea, for example, *Trichodesmium* blooms are mostly confined to the Spring Intermonsoon (SI) period (Qasim, 1970; Devassy et al., 1979; Capone et al., 1998; Poulton et al., 2009; Krishnan et al., 2007). The high surface temperature, calm weather and oligotrophic conditions in surface waters seem to favor growth of *Trichodesmium* during the

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SI season (SenGupta and Naqvi, 1984). In order to study the hitherto unknown potential of *Trichodesmium* in halocarbon production, phytoplankton pigments and halocarbons ($CHCl_3$, CCl_4, CH_2Br_2 and $CHBr_3$) were estimated in the eastern Arabian Sea for one month during this season.

2. Materials and methods

A patchy *Trichodesmium* bloom was observed between 3rd and 5th April 2007 during regular visits to the Candolim Time Series (CaTS) station G3 located in the eastern Arabian Sea off Goa, India (Fig. 1a). In order to investigate the phenomenon in detail and to extend the observations further offshore, three cruises of the coastal research vessel *Sagar Sukti* (SaSu) were undertaken: SaSu 135 from 17th to 18th April., SaSu 139 from 4th to 5th May and SaSu 141 from 10th to 11th May 2007. The bloom extended up to Sta. G8. Monthly composite images of chlorophyll (Chl *a*) and sea surface temperature (SST) prepared with the MODIS data (http://daac.gsfc.nasa.gov/giovanni) are shown in Fig. 2. Niskin bottles (5 L) fixed on polyvinyl chloride (PVC)-coated hydrowire were used for sampling of water for routine measurements as well as for halocarbons and phytoplankton pigments.

2.1. Halocarbons extraction

Subsamples for halocarbons were collected immediately after subsampling for oxygen in 100 ml volumetric flasks by following the standard protocol for dissolved gases, i.e. bubbling was avoided and 200–300 ml of water was allowed to overflow. Triplicate samples were taken during 3rd-5th April but only one sample was collected subsequently from each depth. Samples were preserved immediately at 4 °C in dark until extraction. Halocarbon extraction followed the procedure of Abrahamsson and Klick (1990). For checking the detection limit, water was purged with ultra pure grade nitrogen (purity 99.9995%) and analyzed for any background contamination. Detection limits, which is defined here as signal to noise ratio (S/N=5), were determined by spiking the N_2-purged water with the halogenated compounds being analyzed. The precision was determined as the relative standard deviation of quadruplicate analyses of the spiked samples. The detection limit ranged from 0.01 to 1.2 ng l^{-1} while the precision varied between 7.2 and 9.7%. The lowest detection limit was for the chlorinated compounds ($CHCl_3$ and CCl_4) whereas it was the highest for dibromomethane. Bromoform could be detected at 0.3 ng l^{-1}. A 4–5 point external standard calibration (ESTD) was performed by using pure liquid standards obtained from Sigma Aldrich (Germany). The peak area verses amount of standard (dissolved in methanol (Chromasolv) procured from E. Merck) injected was used to prepare calibration curves. Linearity through the origin was assumed and average calibration factor was taken for quantification. See Roy (2010) for further details.

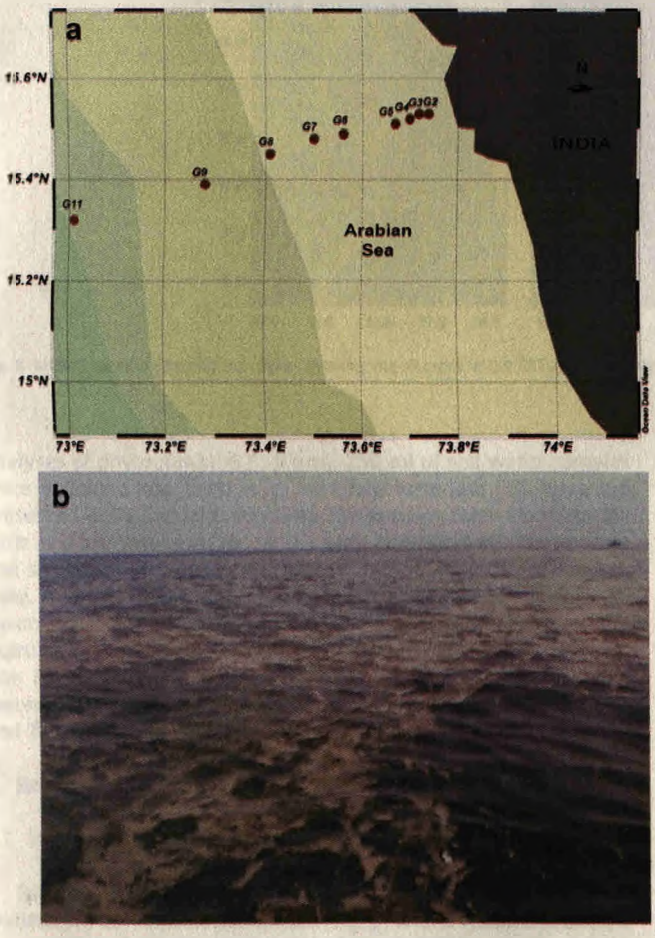

Fig. 1. a) Sampling locations in the eastern Arabian Sea off Goa; b) picture showing patchy *Trichodesmium* bloom observed at CaTS station G3. April.

2.2. Pigments extraction and speciation

For the analysis of phytoplankton pigments, water samples (10–800 ml) were filtered through Whatman GF/F (25 mm, 0.7 μm) filters that were stored at −85 °C until analysis. Pigments were extracted from 3 ml of 100% acetone for 5 min in an ultrasonic bath filled with ice-water. The extracts were stored overnight at −20 °C until analysis. The HPLC analysis was carried out following the method of Roy et al. (2006) however the buffer used was 28 mM tetra butyl ammonium acetate. The method did not separate alpha and betacarotene and hence these compounds were grouped. We also used the weighted sum of different marker pigments as proposed by Utiz et al. (2006) to reconstruct the proportion of various phytoplankton groups for this study. The sum of 19′-hexanoyloxyfucoxanthin (19′HF), 19′-butanoyloxyfucoxanthin (19′BF) and alloxanthin (Allo) was used to indicate nanoflagellates abundance whereas the sum zeaxanthin (Zea) and chlorophyll *b* (Chl *b*) have been used to represent picoplankton group. Microplankton population was represented by the sum of fucoxanthin (Fuco) and peridinin (Perid). It should be noted that the pigment grouping reported here does not strictly represent the true size class and has already been acknowledged by several authors (Vidussi et al., 2001; Roy et al., 2006; Uitz et al., 2006). For calculating the sum of all weighted diagnostic pigments, $\sum$DPw, is expressed as:

$$\sum \mathrm{DPw} = 1.41[\mathrm{Fuco}] + 1.41[\mathrm{Perid}] + 1.27[19'\mathrm{HF}] + 0.35[19'\mathrm{BF}] + 0.60[\mathrm{Allo}] + 1.01[\text{chl } b] + 0.86[\mathrm{Zea}]$$

In comparison to DP, $\sum$DPw represents the chlorophyll *a* concentration, which can be reconstructed from the knowledge of the concentration of the seven other pigments (Uitz et al., 2006). The fractions of the chlorophyll *a* concentration associated with each of the three phytoplankton classes (f_{micro}, f_{nano} and f_{pico}) were then derived according to the following ratios.

$$f_{micro} = (1.41\,[\mathrm{Fuco}] + 1.41[\mathrm{Perid}])/\sum \mathrm{DPw}$$
$$f_{nano} = (1.27[19'\mathrm{HF}] + 0.35[19'\mathrm{BF}] + 0.60[\mathrm{Allo}])/\sum \mathrm{DPw}$$
$$f_{pico} = (1.01[\text{Chl } b] + 0.86[\mathrm{Zea}])/\sum \mathrm{DPw}$$

We used these ratios to derive the proportions of the three groups for our sampling period. For the qualitative and quantitative

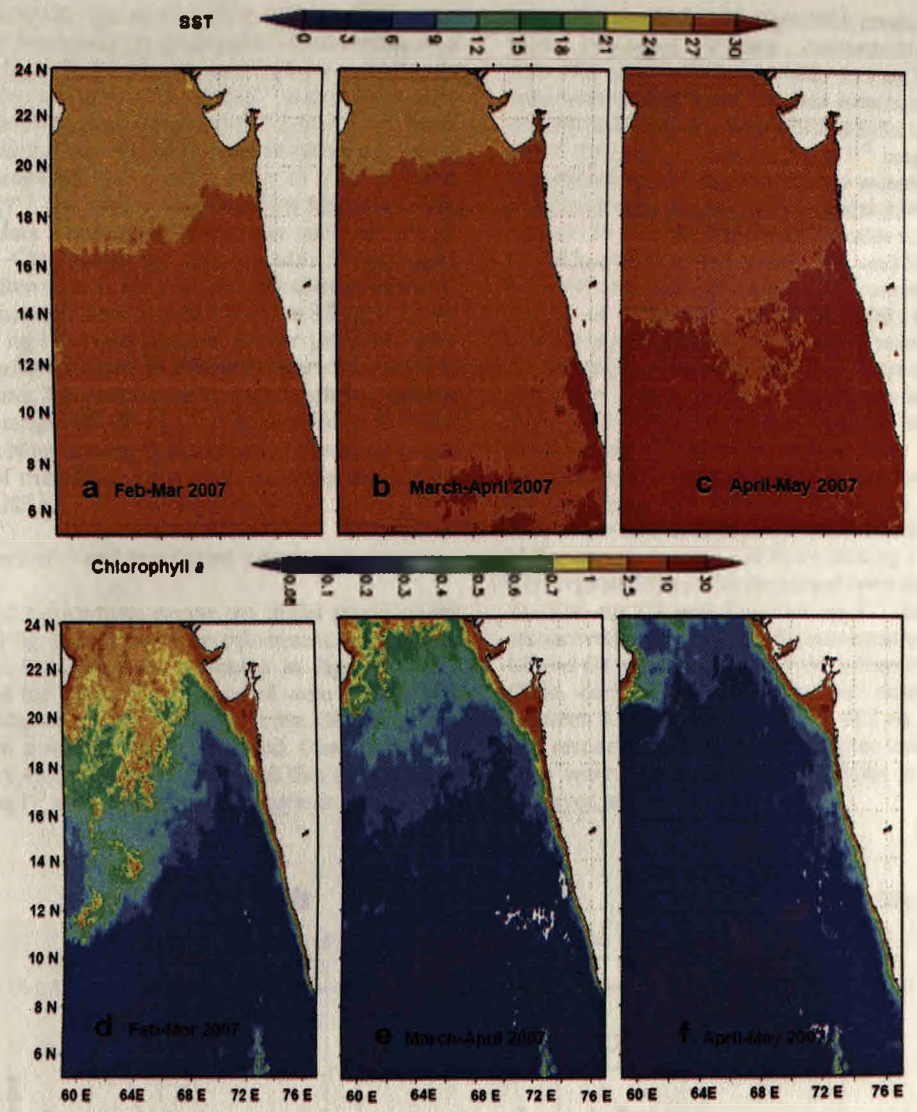

Fig. 2. MODIS satellite observations from (showing the changes in the SST and chlorophyll *a* in the Arabian Sea during the spring intermonsoon season (February–May) of 2007.

analyses of phytoplankton (>5 μm), 250 ml of sea water samples were collected and fixed with acid Lugol's iodine (1% w/v) and preserved in 3% formalin solution. The samples were stored in the dark at a low temperature (4 °C) until enumeration. The settling and siphoning procedure was followed to obtain a 50 ml concentrate. Analyses of this concentrate was carried out in triplicate by mounting 1 ml on a Sedgwick Rafter counting chamber and examining through an Olympus inverted microscope (magnification 100–200×). Generic and species identifications used various taxonomic keys following Subrahmanyam (1959), Lebour (1978) and Tomas (1996).

3. Result

3.1. Variability of phytoplankton pigments

Satellite data for the period February–May 2007 show gradual warming in the central Arabian Sea (Fig. 2) with a systematic decline in the chlorophyll *a* concentration following the cessation of the phytoplankton bloom caused by convective mixing in winter. In general, the SI period (April–May) is characterized by oligotrophic condition (Fig. 2) in the central Arabian Sea which favors the development of cyanobacterial blooms such as the *Trichodesmium* bloom that we observed off the central west coast of India during April–May 2007. The waters affected by the bloom were distinguished by high concentrations of marker pigments zeaxanthin (23 μg l^{-1}), alpha and betacarotene (6 μg l^{-1}) and chlorophyll *a* (67 μg l^{-1}) (Fig. 1a). Apart from *Trichodesmium*, other phytoplankton groups, especially diatoms, were also present in large numbers at depths below the bloom. The major diatom species at G3 were *Bacteriastrum furcatum* (range 0–10,500 cells l^{-1}), *Chaetoceros curvisetus* (5456–24,173 cells l^{-1}) and *Leptocylindrus minimus* (range 11000–25000 cells l^{-1}). Some dinoflagellates such as *Gymnodium* spp (range 496–2832 cells l^{-1}) were also encountered. Slightly offshore (at Sta. G5) chlorophyll *a* and zeaxanthin concentrations were much lower than those at Sta. G3. Levels of other pigments more or less remained constant. Fucoxanthin and zeaxanthin decreased significantly from the first observation to the second with a marginal decrease thereafter. While peridinin concentrations were higher during SaSu 139, the marker pigments for nanoflagellates

were lower during SaSu 141. The results clearly indicate the decay of the bloom with time. The lowest concentrations of zeaxanthin were measured at the bottom depths of outermost stations sampled (G9 and G11). Fucoxanthin ranged from 0 to 58 ng l^{-1} with a mean value of 9 ng l^{-1}. Higher concentrations of fucoxanthin generally occurred at Stas. G2–G6 indicating that the bloom was most intense in the shallow waters. Zeaxanthin had a mean value of 5 ng l^{-1} with a range of 0–62 ng l^{-1}, the higher concentrations being generally associated with surface samples collected from stations farthest from the coast (G7–G11) during the decline phase of the bloom. Peridinin, which is often used as a marker pigment of dinoflagellates, also occurred in concentrations ranging from 0 to 18 ng l^{-1} with a mean value of 6 ng l^{-1}. Nanoflagellate marker pigments were significantly high during the bloom in subsurface waters but showed a gradual decrease later. The mean value for nanoflagellate pigments was 7 ng l^{-1} with a range of 0–59 ng l^{-1}. In general, concentrations of chlorophyll *a* and other marker pigments varied over large ranges (over two orders of magnitude even after excluding the highest values found at Sta. G3.)

3.2. Changes in chlorinated and brominated halocarbons

Depth profiles of halocarbons during the initial phase of the bloom are shown in Fig. 3 while data from subsequent cruises (SaSu 135, SaSu 139 and SaSu 141) are presented in Figs. 4–6. The concentrations of all the halocarbons measured were initially high and decreased rapidly with time. However, at Sta. G8 brominated compounds showed a marked increasing trend. Concentration of $CHCl_3$ ranged from 0.45 to 97.92 ng l^{-1} whereas that of CCl_4 varied from 0.04 to 3.84 ng l^{-1} with the high concentrations of CCl_4 being generally associated with high $CHCl_3$ levels. Both of these compounds were measured in low concentrations during SaSu 135 (0.6–17.5 ng l^{-1} $CHCl_3$ and 0.02–1.13 ng l^{-1} CCl_4), and the highest value was recorded from surface waters of Sta. G4. Similarly $CHCl_3$ concentrations during SaSu 139 and SaSu 141 were within the ranges of 0.7–25.2 ng l^{-1} and 0.4–35.1 ng l^{-1}, respectively, with the highest concentrations coming from surface waters at Sta. G7 which was also associated with slightly lower temperature (28.5–30.2 °C) and lower salinity (35.5–35.8). There was a notable similarity in the distribution of $CHCl_3$ and CCl_4 during SaSu 135 and SaSu 139. Similarly during SaSu 139 and SaSu 141 cruises CCl_4 concentrations were within the ranges of 0.02–0.82 and 0.03–1.06, respectively with lower concentrations generally occurring in near-bottom waters.

The highest concentrations of brominated methanes (CH_2Br_2 and $CHBr_3$) were also found during the initial phase of the bloom (Figs. 3–5). CH_2Br_2 at the inpatch location had a range of 3.4–35.3 ng l^{-1} whereas during SaSu 135, it had a range of 2.0–36.0 ng l^{-1}. Similarly $CHBr_3$ had a range of 10.7–16.7 ng l^{-1} at the inpatch station. Although station G5 was not under the influence of the bloom $CHBr_3$ concentrations found here were relatively higher and had a range of 15.0–30.4 ng l^{-1}. Brominated methanes (CH_2Br_2 and $CHBr_3$) also decreased with time. CH_2Br_2 had a range of 1.1–37.6 ng l^{-1} and 2.9–23.6 ng l^{-1} during SaSu 139 and 141 respectively with the highest concentrations being recorded at Stas. G8 and G9 which were under the influence of decaying bloom. The $CHBr_3$ concentration also showed similar distribution ranging between 1.3–16.1 ng l^{-1} and 2.2–9.7 ng l^{-1} during SaSu 139 and 141, respectively. Interestingly, higher values of brominated methanes were also associated with high zeaxanthin concentrations observed at Stas. G8 and G9.

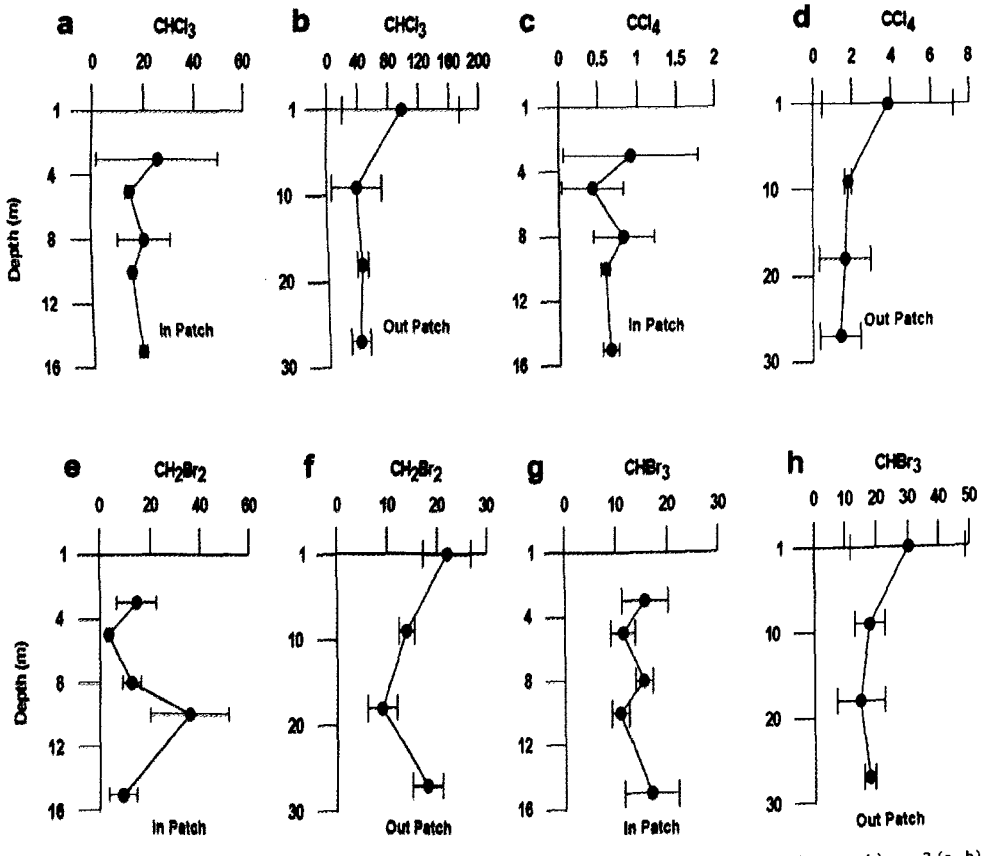

Fig. 3. Comparison between halocarbons depth profiles observed at CaTS stations G3 (inpatch) and G5 (out patch) $n = 3$ (a–h).

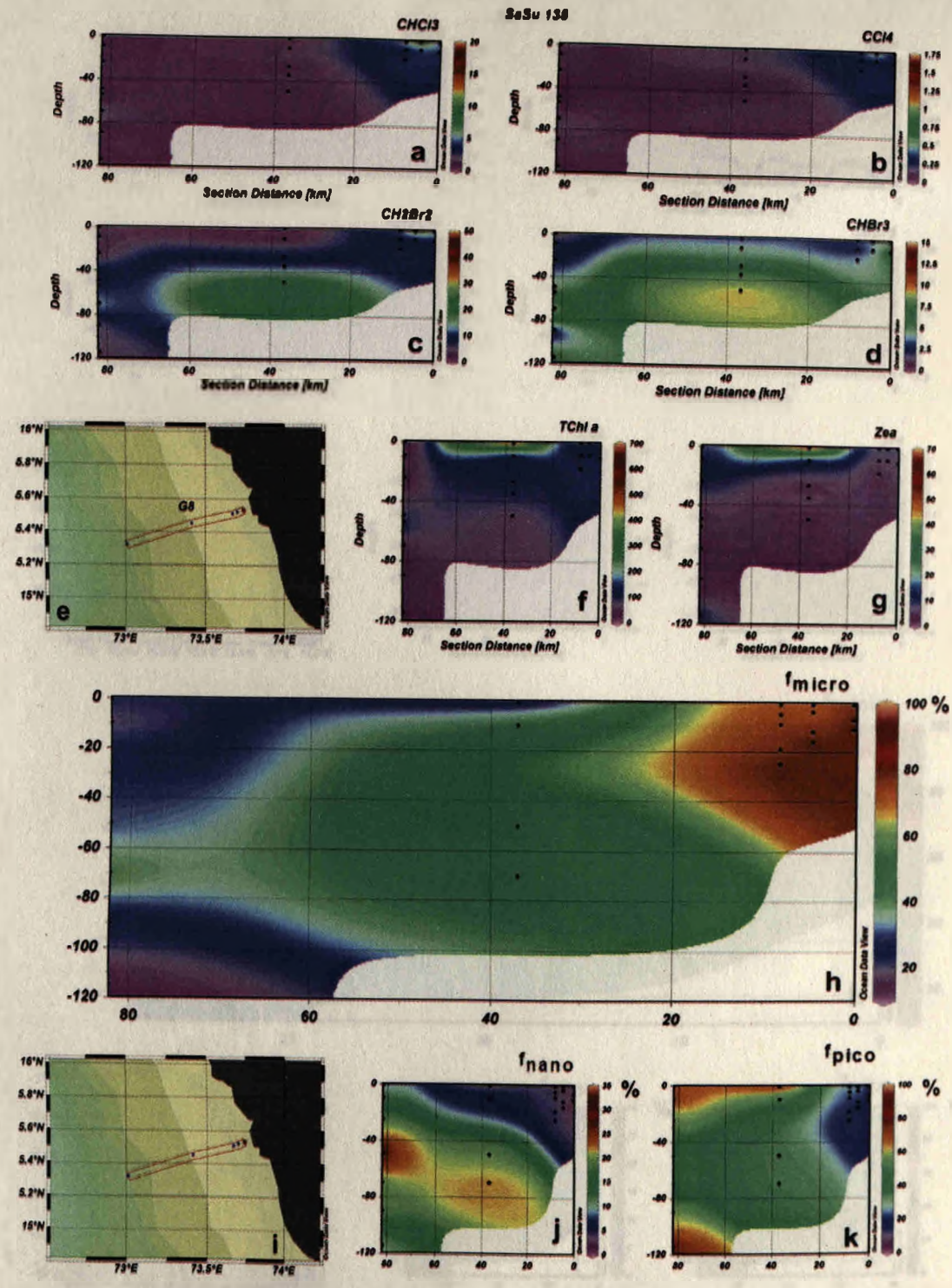

Fig. 4. Sampling sections and distribution of halocarbons and phytoplankton pigments in (ng l^{-1}) off Goa during the cruise SaSu 135.

4. Discussion

4.1. General characteristic and variability of pigments

Surface water in the study region experienced warming between April and May resulting in strong vertical stratification (Fig. 2). The resultant restricted supply of nutrients from the thermocline to the surface water led to oligotrophic conditions. However, although dissolved inorganic nitrogen concentrations were often below the detection limits, phosphate was never completely depleted. This together with relatively calm weather favors the growth of diazotrophs, especially *Trichodesmium* (Devassy et al., 1978; SenGupta and Naqvi, 1984). Microscopic observations confirmed the presence of *Trichodesmium erythraeum* in our area of study and also showed

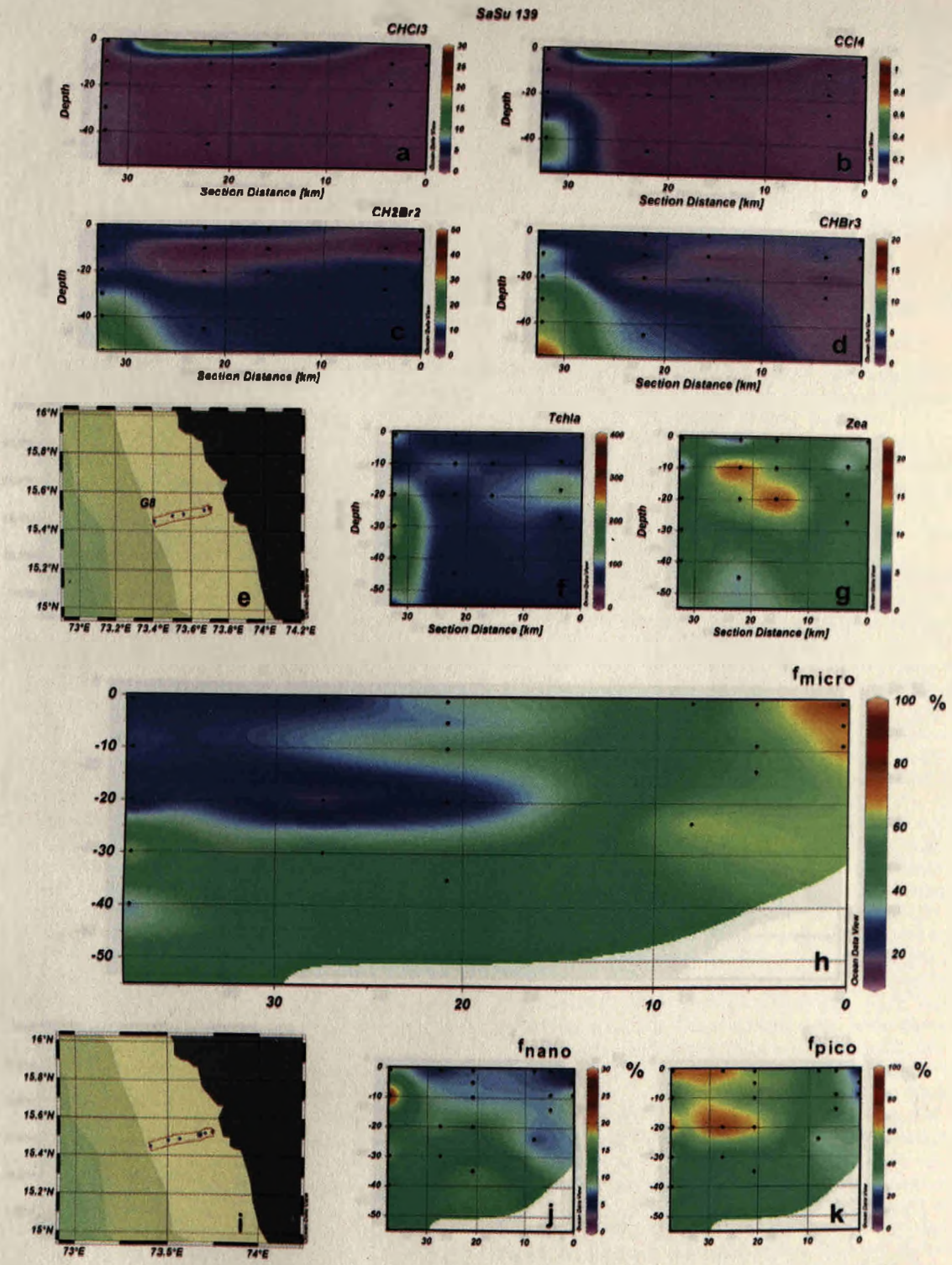

Fig. 5. Sampling sections and distribution of halocarbons and phytoplankton pigments in (ng l^{-1}) off Goa during the cruise SaSu 139.

significant bacterial associations with the trichomes. Such associations are well documented (Siddiqui et al., 1992; Villareal, 1992; Nausch, 1996; Mulholland, 2007). We also used phytoplankton pigment data to understand the phytoplankton composition changes during our month-long investigation. In general, Chl *a* biomass was low during the entire period except in waters that were affected by the bloom (see Figs. 4–6). Zeaxanthin was the most abundant marker pigment. The highest concentrations of this pigment were observed in both the surface and subsurface waters at Sta. G8. At this station fucoxanthin and peridinin were also found at shallow depths (14–25 m), suggesting the presence of larger plankton such as diatoms and dinoflagellates (Figs. 4–6). Significantly, fucoxanthin

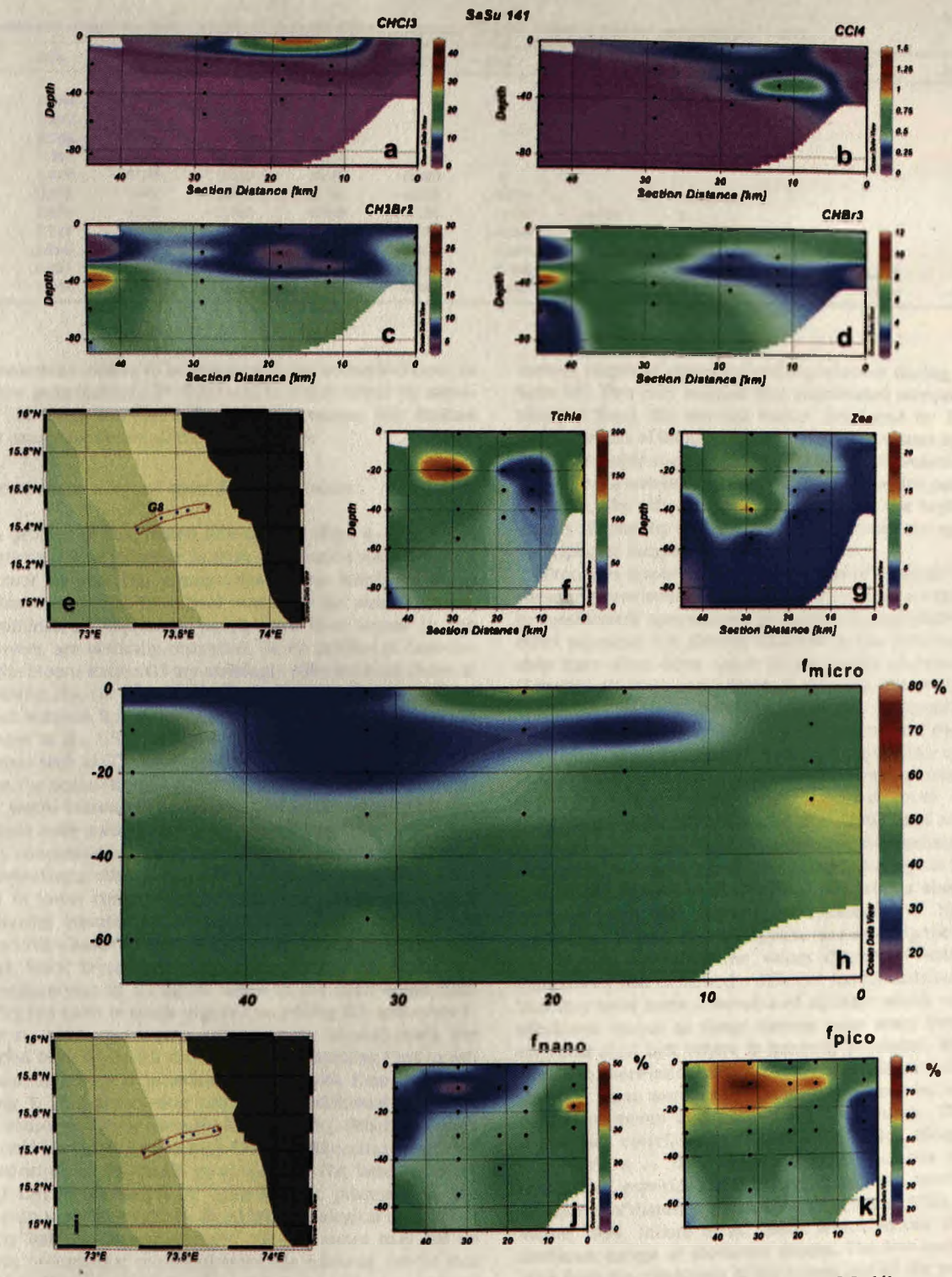

Fig. 6. Sampling sections and distribution of halocarbons phytoplankton pigments in (ng l^{-1}) off Goa during the cruise SaSu 141.

concentrations exhibited a systematic increasing trend with time, especially at Stn G8 that was under the influence of the bloom. This could be related to the release of fixed nitrogen to the water column from the bloom, particularly in its decline phase, which would promote the growth of larger phytoplankton. Such observations have also been reported from Gulf of Mexico (Mulholland et al., 2006).

Concentrations of 19′ HF and 19′ BF pigments were low during our investigation; therefore, much of the fucoxanthin content was presumably from diatoms and not from prymnesiophytes. It would appear that the contribution of nanoflagellates to chlorophyll biomass was mostly from alloxanthin-contaning cryptophytes. Diagnostic pigment indices computed based on Utiz et al. (2006)

Table 1
Pearson correlations analysis between halocarbons and phytoplankton pigments ($n = 66$). Values in bold are significant at $\alpha = 0.05$.

	$CHCl_3$	CCl_4	CH_2Br_2	$CHBr_3$	Temp	Oxy	Sal	Chl *a*	Zea	Per	Fuco	Allo
$CHCl_3$	1											
CCl_4	**0.940**	1										
CH_2Br_2	**0.257**	**0.285**	1									
$CHBr_3$	**0.764**	**0.764**	**0.324**	1								
Temp	0.081	0.058	0.147	0.031	1							
Oxy	0.079	0.079	0.157	0.032	**0.483**	1						
Sal	−0.078	−0.049	0.077	0.186	**−0.338**	−0.056	1					
Chl *a*	0.090	0.052	0.087	**0.248**	0.230	0.159	−0.151	1				
Zea	0.123	0.088	0.133	**0.281**	0.159	−0.064	−0.139	**0.675**	1			
Per	0.066	0.041	0.080	−0.008	**0.269**	0.089	−0.207	0.029	−0.054	1		
Fuco	0.097	0.074	−0.113	0.132	0.135	**0.306**	−0.069	**0.580**	0.075	**−0.287**	1	
Allo	−0.204	−0.164	−0.101	0.000	**−0.286**	−0.093	**0.496**	−0.111	−0.076	−0.056	−0.124	1

showed nearshore waters to be dominated by microplanktons. In contrast the picoplankton (70–80%) and to lesser extent by nanoplankton (30–20%) dominated the offshore waters. This feature remained consistent during all the three cruises.

4.2. Halocarbons sources and sinks during the bloom

Depth profiles of chlorinated halocarbons (Figs. 4–6) suggest strong anthropogenic influence as their distribution was restricted to shallower depths. The strong thermocline which develops during this season inhibits vertical mixing of the water column. Thus, brominated compounds, which have their source in the deeper layers, are vertically restricted. Depth profiles of halocarbons at the bloom station G3 are strikingly different from those at the adjoining Sta. G5 (Fig. 3), with the former showing minor subsurface maxima. It is of note that concentrations of chlorinated halocarbons at Sta. G5 were much higher than those at Sta. G3. Halocarbons such as CCl_4 have often been used as a tracer of water masses in the ocean (Krysell, 1992; Krysell et al., 1994; Fogelqvist, 1985). It seems reasonable to assume that water masses that the two stations were quite different.

$CHBr_3$ concentrations recorded at the bloom station G3 was much lower than at other stations. In general, we found that $CHBr_3$ occurred in lower concentrations relative to CH_2Br_2. In coastal waters having elevated concentrations of $CHBr_3$, the ratio of CH_2Br_2 to $CHBr_3$ has often been found to be 0.1 or less (Moore and Tokarczyk, 1993; Krysell and Nightingale, 1994; Carpenter et al., 2003; Abrahamsson et al., 2004) while in the open ocean with low $CHBr_3$ the ratio is much higher (exceeding 0.5 and even 1; Butler et al., 2007; Quack et al., 2007). In the present study, the ratio varied from 0.3 to 0.9 during the first sampling (3rd to 5th April) and thereafter it increased further with time to values exceeding 1. This observation calls for an additional source of CH_2Br_2, and/or relatively stronger loss of $CHBr_3$. Other processes which could also give rise to a high CH_2Br_2:$CHBr_3$ ratio have been described in detail by Quack et al. (2007). The latter workers reported CH_2Br_2 formation by heterotrophic processes in the Mauritanian upwelling system. In addition, biological removal of $CHBr_3$ by bacteria associated with the trichomes may be an important process that could influence the turnover rate of this compound. Thus, our results are consistent with observations from the Mauritanian upwelling system and highlight multiple sources of CH_2Br_2 formation and/or $CHBr_3$ degradation by bacteria associated with the trichomes.

Even though there occurred a considerable decrease in halocarbon concentrations during the later phase of the bloom, the average water column concentrations of brominated compounds at Sta. G8 exhibited an increasing trend. This is the same location where the bloom appeared later and could be observed under various stages of development/degradation during SaSu 135 and SaSu 141. This may suggest that brominated compounds could be released from the detrital matter produced by *Trichodesmium*. Recent results of incubations of marine aggregates by Hughes et al. (2008) strongly suggest the release of several volatile halocarbons.

There is a notable similarity of the distribution patterns of $CHCl_3$ and CCl_4. The other noteworthy feature is the high variability in waters affected by the bloom as evident from the spread of values for triplicate samples in the surface layer.

Processes responsible for production of biogenic halocarbons in the marine environment are very complex and are therefore subject to considerable speculation. Although it is improbable that Chl *a* or other pigments are directly involved in the halocarbon synthesis, they have often been taken as surrogates of processes involved (Schall et al., 1997 and references therein). We performed Pearson correlation analysis between trace gas concentrations, phytoplankton pigments and hydrographic parameters measured during the bloom (Table 1). $CHCl_3$ showed a strong positive correlation with CCl_4 ($r^2 = 0.94$, $p = 0.05$, $n = 66$). High concentrations of chlorinated halocarbons have been often reported from coastal areas (Nightingale et al., 1995; Christof et al., 2002) and are attributed to production by macroalgae or anthropogenic discharge. The macroalgal growth along the Indian west coast during the SI is negligible, and so the source of chlorinated halocarbons should be mostly anthropogenic. The highest concentrations of the brominated compounds CH_2Br_2 and $CHBr_3$ were found during the initial phase of the bloom; thereafter the values decreased with time. These compounds also exhibited a different spatial distribution pattern in that they were more concentrated offshore which suggest a strong planktonic source as these stations were away from the mostly-intertidal algal belt where is naturally produced. Also, as pointed out above, there is not much growth of macroalgae during this period. However, there were no significant correlations between any of the marker pigments and brominated compounds. The chlorinated compounds correlated positively with $CHBr_3$, though ($r^2 = 0.76$, $p = 0.05$, $n = 66$). Enhanced concentrations of brominated compounds, especially $CHBr_3$, have often been associated with the abundance of diatoms (Klick and Abrahamsson, 1992; Tokarczyk and Moore, 1994; Moore et al., 1996), which in our study were not dominant except at shallower depths. The low correlations could arise from the patchiness of the bloom and/or the presence of high bacterial associations, which could have influenced the turnover rates of these compounds.

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Identification of non-indigenous phytoplankton species dominated bloom off Goa using inverted microscopy and pigment (HPLC) analysis

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An unusual phytoplankton bloom dominated by unidentified green coloured spherical algal cells (~5 μm diameter) and dinoflagellates (*Heterocapsa*, *Scripsiella* and *Gymnodinium*) was encountered along the coast of Goa, India during 27 and 29 January, 2005. Pigment analysis was carried out using both fluorometric and HPLC methods. Seawater samples collected from various depths within the intense bloom area showed high concentrations of Chl *a* (up to 106 mg m^{-3}) associated with low bacterial production (0.31 to 0.52 mg C m^{-3} h^{-1}) and mesozooplankton biomass (0.03 ml m^{-3}). Pigment analyses of the seawater samples were done using HPLC detected marker pigments corresponding to prasinophytes, dinoflagellates and diatoms. Chlorophyll *b* (36–56%) followed by peridinin (15–30%), prasinoxanthin (11–17%) and fucoxanthin (7–15%) were the major diagnostic pigments while pigments of cryptophytes and cyanobacteria including alloxanthin and zeaxanthin formed <10%. Although microscopic analysis indicated a decline in the bloom, pheaophytin concentrations in the water column measured by both techniques were very low, presumably due to fast recycling and/or settling rate. The unique composition of the bloom and its probable causes are discussed in this paper.

1. Introduction

Coastal and near-shore waters are more productive regions in the marine environment nourished by nutrients derived by regeneration, upwelling and land run-off. Phytoplankton blooms in these waters follow a seasonal pattern that shapes the coastal marine ecosystem. Environmental forcing, nutrient availability, predator communities and land-driven inputs are the major factors that control coastal and near-shore phytoplankton community and blooms. Several anthropogenic factors in these environments can result in alteration of coastal water quality (eutrophication), introduction of non-native species (ballast action), alteration of predator community (over-fishing), etc. As a result, non-periodic and exceptional blooms of both noxious and/or toxic phytoplankton species lasting for few weeks to months are frequently reported, influencing the seasonal patterns of dominant phytoplankton community structure, thereby affecting coastal and estuarine biogeochemical processes (Cloern 1996).

Keywords. Dinoflagellates; prasinoxanthin; HPLC; pigment composition; phytoplankton bloom.

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The primary production and phytoplankton community in the waters along the coast of India are largely influenced by monsoonal forcings, resulting in changes in the coastal currents, surface temperatures, nutrient availability, etc. For example, Banse *et al* (1996) hypothesized increased nutrient supply as principal factor controlling primary production in the western coastal waters of India. Along with nutrient injection in the coastal waters, seasonal changes in the surface coastal current directions (Shetye *et al* 1994) coupled with changes in salinity and temperature regulate the phytoplankton diversity along west coast of India (Qasim 1977). Phytoplankton diversity, blooms and their life stages have been exhaustively studied in these waters following the classical microscopy methods (Subrahmanyan 1958; Dehadrai and Bhargava 1972; Devassy *et al* 1978; Sawant and Madhupratap 1996), remote sensing (Desa *et al* 2005; Gomes *et al* 2008), molecular techniques (Godhe *et al* 2001) and of late using pigment identification by HPLC technique (Roy *et al* 2006; Parab *et al* 2006; Roy 2010). All these studies show that diatoms are the pre-dominant forms of phytoplankton followed by seasonal blooms of dinoflagellates and *Trichodesmium*.

In the recent past, 'unusual blooms' of phytoplankton species unrelated to the seasonally recurring communities have been reported. Some of these blooms have been either toxic (Sahayak *et al* 2005), caused nuisance to coastal population (Ramaiah *et al* 2005) or led to discolouration of coastal waters (Nayar *et al* 2001). Moreover, the frequency of these blooms in the near-shore waters of the west coast of India has increased which reportedly coincide with increased commercial shipping in these waters (Anil *et al* 2002). One common trait in all these blooms – harmful or otherwise was their sporadic nature; most of them were never encountered earlier and their occurrences in these waters were largely uncommon. Here we report one such occurrence of phytoplankton bloom in the near-shore waters of Goa, west coast of India dominated by cells hitherto not observed in these waters. Moreover, an attempt is made to explain possible cause of this bloom in these waters.

2. Material and methods

2.1 *Study site and sampling*

The mixed phytoplankton bloom was observed during one of the monthly sampling at the Candolim time series (CaTS) transect (figure 1) in the near-shore waters off Goa, west coast of India. Seawater was sampled on two days (27 and 29 January,

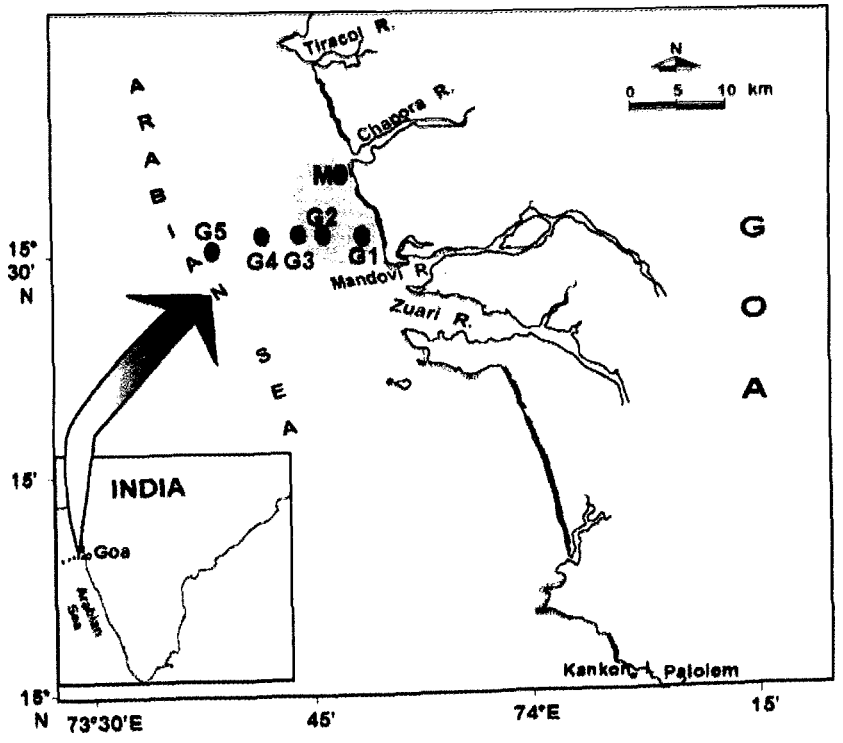

Figure 1. Map showing the CaTS (Candolim time-series) stations G1 to G5 and one station off Morjim north of CaTS. The approximate spread of the bloom is indicated by the shaded portion.

2005). On the first day of sampling, seawater from three stations (G1, G2, G3) along the CaTS transect (table 1) were sampled. On the subsequent day, sampling was restricted to G1, G2 and station off Morjim (M) (figure 1).

Seawater was collected using a 5 litre Niskin sampler and sub-samples were collected for phytoplankton enumeration and its generic composition, chlorophyll *a* (Chl *a*), pigment composition and bacterial production.

2.1.1 *Chl a and other pigments*

Aliquots of seawater were filtered on GF/F (0.7 µm) filters for chlorophyll *a* and pigment composition. The Chl *a* filters were extracted immediately after filtration whereas pigment samples were stored in liquid nitrogen prior to analyses. Both Chl *a* and pigment samples were extracted in 90% acetone under refrigeration.

2.1.2 *Phytoplankton abundance*

Samples for phytoplankton abundance and composition were fixed with Lugol's iodine and stored in dark prior to microscopic examination.

2.1.3 *Bacterial production*

Ten millilitre of unfiltered samples were spiked with 20 µl of $^3H_1$-thymidine (1 mCi; specific acitivity: 12,000 mCi mmol^{-1}) in triplicates and incubated for 60 minutes in the dark in ambient conditions. The uptake was stopped by adding 0.2 µm filtered formalin (4% final concentration). Formalin prefixed samples were used as controls and were incubated similarly.

Additional sample for mesozooplankton biomass was collected using towing method (HT net, 200 µm mesh) and the samples were preserved in 4% formalin. All the samples were brought back to the laboratory within 3 hours of sampling for further processing and were stored in ice during transport.

2.2 *Analyses*

Chlorophyll *a* (Chl *a*) and phaeophytin concentrations were measured fluorometrically on a Turner fluorometer (UNESCO 1994). For estimation of Chl *a*, the filter paper containing the sample was crushed and the pigments were extracted in 10 ml of 90% acetone in dark for 24 h in the refrigerator. After extraction, the fluorescence was measured before (F_o) and after acidification (F_a) with two drops of 1.2 N HCl. The actual concentrations of Chl *a* and phaeophytin pigments were then calculated following JGOFS protocol (UNESCO 1994).

The total pigment samples were analyzed following a slightly modified method described by van Heukelem (2002) as described by Roy *et al* (2006). The HPLC system was equipped with an Eclipse XDB C-8 reverse phase column (4.6 × 150 mm) attached to a guard column, an Agilent 1100 pump, online degasser and diode array detector (Agilent Technologies Model 1100). The column was maintained at 60°C and the elution was carried out using a linear gradient program over 36 min at the rate of 1.1 ml min^{-1}. The eluant was a mixture of solvent A (methanol (70:30) + 1 M ammonium acetate (pH 7.2)) and solvent B (methanol). The solvents A and B were mixed from 95/5% to 5/95% for the first 22 min followed by an isocratic separation with 95% solvent B up to 30 min to elute α- and β-carotenes. The column was equilibrated for 6 min between two successive analyses. The pigments were detected using a UV-diode-array detector. Pigment standards (DHI Waters. Denmark and Sigma-Aldrich, USA) for Chl *a* and 15 different marker pigments were used to identify and quantify various phytoplankton groups. The marker pigments were chlorophyll c_2 (Chl c_2),

Table 1. *Distribution of chlorophyll a, phaeophytins and total bacterial production (BP) during the bloom period in the near shore waters off Goa.*

	27/01/2005							29/01/2005				
	G1		G2		G3			G1		G2	Off Morjim	
Location	73.45.6°E 15.31.2°N		73.44.4°E 15.31.2°N		73.43.8°E 15.31.2°N			73.45.6°E 15.31.2°N		73.44.4°E 15.31.2°N	73.43.4°E 15.37.4°N	
Parameters	1 m	4 m	1 m	9 m	1 m	7 m	14 m	1 m	4 m	1 m	1 m	6 m
Chlorophyll *a*	106.8	3.2	17.6	1.4	6.5	3.4	0.7	22.9	11.7	19.8	18.7	3.8
Phaeophytins	0.84	0.37	0	0.73	0	0.1	0.73	0	0	0.4	0.3	1.1
BP	nd	nd	nd	nd	nd	nd	nd	0.52	0.33	nd	0.31	0.34

Chlorophyll *a* and phaeophytins concentrations are in mg m^{-3}; bacterial production (BP) is in mg-C m^{-3} h^{-1}.
nd: not done.

chlorophyll c_3 (Chl c_3), peridinin (Per), fucoxanthin (Fuc), 19' butanoyloxyfucoxanthin (19' BF), neoxanthin (Neo), violaxanthin (Viola), prasinoxanthin (Pra), alloxanthin (Allo), 19' hexanoyloxyfucoxanthin (19' HF), diadinoxanthin (Diad), zeaxanthin (Zea), lutein (Lut), chlorophyll *b* (Chl *b*), total carotene ($\alpha + \beta$, Tcar), chlorophyll *a* (Chl *a*) and phaeophytin (Phaeo *a*). Diagnostic pigment (DP) concentrations were calculated as described by Roy *et al* (2006) after including Pra as prasinoxanthin also contributed significantly to the pigment concentration. The standards for carotenes (α- and β-types) were purchased individually from DHI Waters and calibrated as a mixture. Both forms of carotenes had similar elution time and could not be resolved properly. Therefore, the two carotenes were grouped together and expressed as Tcar. All the other chemicals used were procured from Merck (Germany).

The samples collected for phytoplankton composition and enumeration were examined following sedimentation technique using Utermohl's inverted microscope (Hasle 1978).

The samples for bacterial production was filtered onto 0.2 μm cellulose nitrate filters, extracted in cold trichloroacetate (TCA), the paper dissolved in scintillation cocktail (UNESCO 1994) and activity measured using a scintillation counter (Wallac model 2000). Zooplankton biomass was estimated by volume displacement method (Madhupratap and Haridas 1990).

3. Results

On day 1 of sampling, the sampled waters at G1 and G2 were dirty brown in colour compared to surface waters outside the patch (figure 2). The patch was tracked up to Morjim and appeared to be concentrated in near shore waters. A similar distinct horizontal and vertical spatial variation was also observed in all the major parameters (table 1). The surface Chl *a* concentration decreased from 106.8 mg m^{-3} at G1 to 6.5 mg m^{-3} at G3. A similar decrease with depth was also observed at all stations (table 1). The depth and spatial profile of Chl *a* indicated that the bloom cells were concentrated close to the shores. During the subsequent sampling, the surface Chl *a* value at G1 dropped to 22.9 mg m^{-3} with a substantial increase in near bottom chlorophyll concentrations to 11.7 mg m^{-3}. The spatial spread of the bloom up to Morjim was evident as the surface Chl *a* concentrations remained nearly same. The phaeophytin concentration after acidification of Chl *a* samples was always less than 1 mg m^{-3} during the sampling.

HPLC analysis of the pigment samples detected a range of diagnostic pigments (DP) specific for dinoflagellates. prasinophytes, diatoms, cyanobacteria and cryptophytes (figure 3). Unlike the fluorometric method, the HPLC method measured low Chl *a* concentrations (4.1–6.5 mg m^{-3}) in the surface waters at G1 and Morjim. Based on the DP calculation, the bloom was dominated by Chl *b* (36–56%), peridinin (15–30%), prasinoxanthin (11–17%) and fucoxanthin (7–15%) (figure 3). The concentration of the prasinoxanthin was higher in surface waters compared to other pigments. Other DP including diadinoxanthin, zeaxanthin, alloxanthin, etc., contributed <5%, respectively.

Microscopic observations showed that the bloom was dominated by unidentified unicellular green coloured algal cells during both days of sampling (figure 4). On the first day of sampling, the bloom cells completely covered the field area and formed aggregates. The cells were covered with mucus

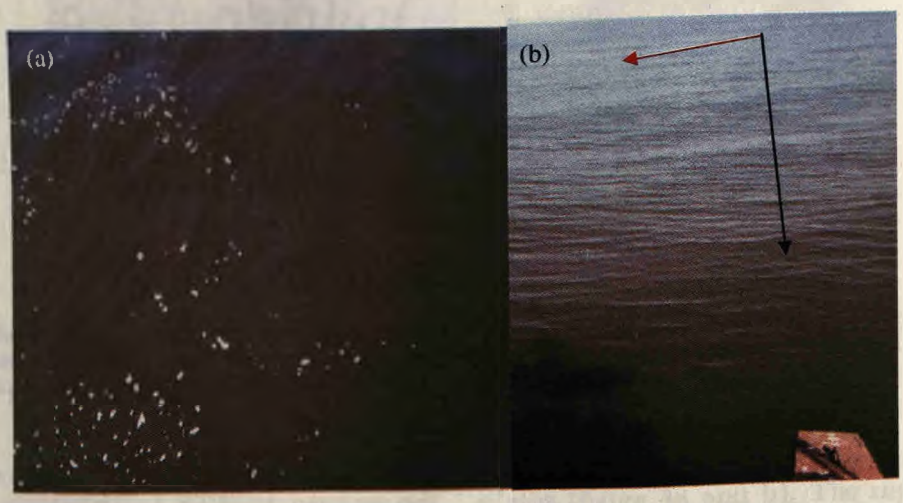

Figure 2. Photographs of (a) surface waters from within the bloom patch showing distinct muddy colour and (b) edge of the bloom patch showing different colours outside (red arrow) and inside (black arrow) the bloom patch.

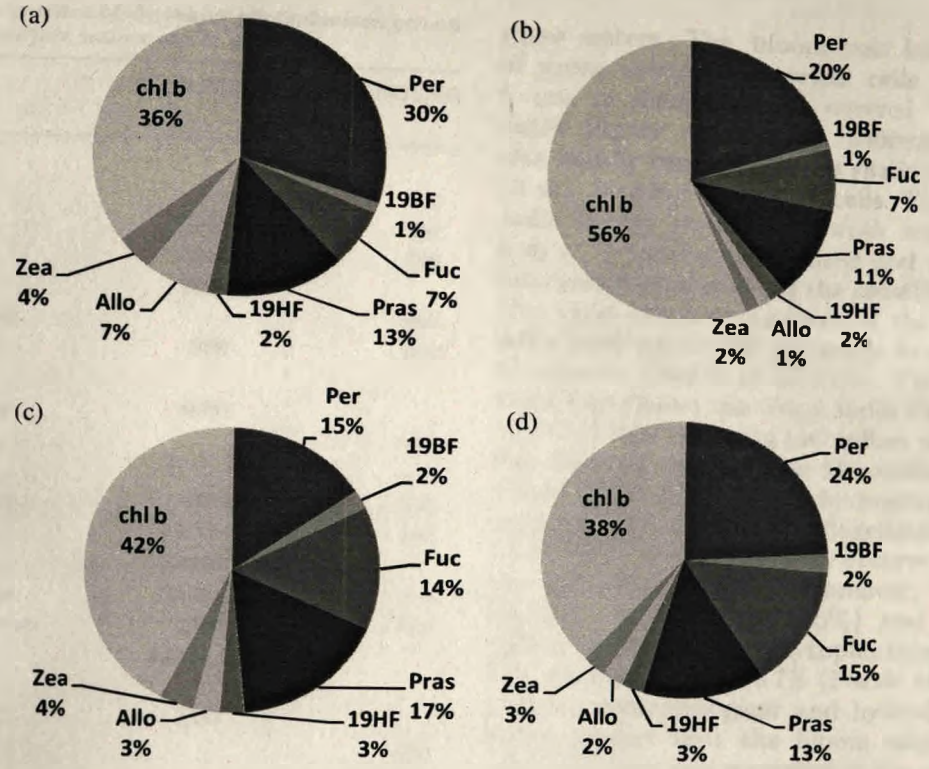

Figure 3. Percent distribution of major diagnostic pigments identified using HPLC method in samples collected from (**a**) G1 surface, (**b**) G1 4 m, (**c**) Morjim surface, and (**d**) Morjim 6 m.

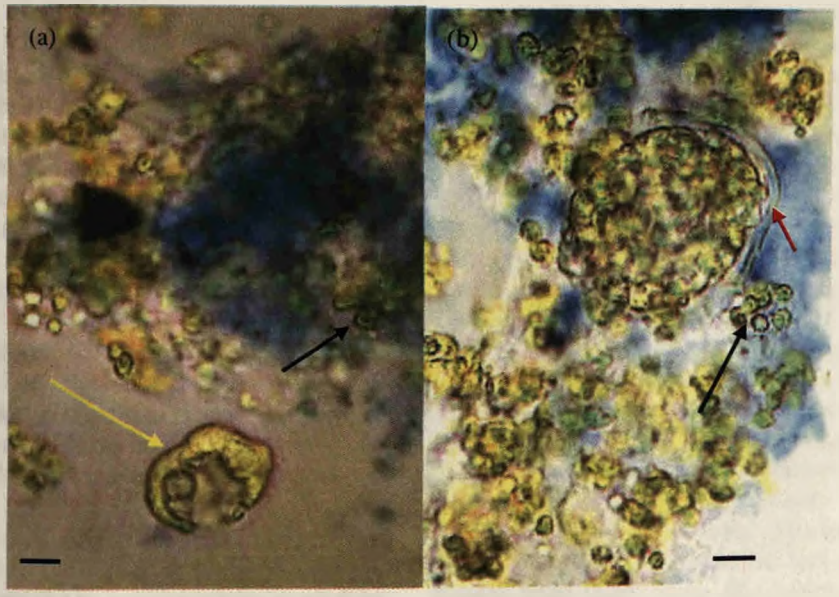

Figure 4. Microphotographs of the bloom sample showing (**a**) broken dinoflagellates cell (yellow arrow) and (**b**) an intact dinoflagellate containing green unidentified cells (red arrow). Also note the unidentified green cells and the mucus stained with alcian blue in the background (black arrows). Scale in each photograph equals 10 μm.

which could be stained with Alcian blue. The cells could not be disaggregated without causing cell breakage and hence were difficult to count. The samples contained broken as well as intact green coloured unidentified algal cells as well as dinoflagellates (figure 4a and b). The ruptured cell-walls of algae as observed microscopically suggested that many of these cells were in a state of decay and

Table 2. *The composition of the major phytoplankton genera identified in the surface waters at G1 station.*

Phytoplankton composition	27/01/2005	29/01/2005
Diatoms		
Amphora sp.	—	300
Navicula sp.	8000	200
Nitzschia sp.	1333	200
Coconeis sp.	2000	
Coscinodiscus sp.	—	800
Pleurosigma sp.	800	100
Dinoflagellates		
Amphidinium sp.	4000	—
Ceratium sp.	—	600
Dinophysis sp.	—	100
Gymnodinium sp.	8000	200
Gyrodinium sp.	12000	200
Heterocapsa sp.	34667	—
Prorocentrum sp.	38666	400
Protoperidinium sp.	4000	500
Scrippsiella sp.	145333	—
Silicoflagellates		
Dictyocha sp.	1333	—
Distephanus sp.	—	200
Unidentified algal cells	NC	1150000
Total cells L^{-1}	263866	1153800

NC: Not counted.

the bloom was in the declining phase. The surface samples collected subsequently showed that the unidentifiable cells amounted to ~99% of total phytoplankton abundance (~1.15×10^6 cells l^{-1}; table 2). Phytoplankton cells other than the bloom cells decreased from 2.6×10^5 cells l^{-1} on the first day of sampling to 0.04×10^5 cells l^{-1} during the subsequent sampling. Among these phytoplankton cells, dinoflagellates constituted ~93.5% (2.4×10^5 cells l^{-1}) on the first day of sampling. During the second day of sampling, the contribution of dinoflagellate decreased to ~56%. *Heterocapsa* sp., *Scripsiella* sp. and *Gymnodinium* sp. dominated the dinoflagellates community (table 2). Diatoms formed <4% of the total phytoplankton population.

Bacterial production rates were low and ranged from 0.31 to 0.52 mg C m^{-3} h^{-1} (table 1). Copepods formed the bulk of the mesozooplankton population (>200 μm) and the total mesozooplankton biomass was 0.03 ml m^{-3}.

4. Discussion

A mixed phytoplankton bloom at the CaTS stations was a chance observation and unique for these waters. The bloom was largely composed of green coloured spherical cells which were 4–5 μm in diameter and covered in mucus exudates (figure 4a and b). Moreover, the bloom was mainly confined to the shallow waters (depth 12 m), forming clumps of cells. The west coast of India largely experiences weak sea-breeze (1.5 to 5 m s^{-1}) between November and April and wind direction is almost along the coastline at the CaTS. The cross shore component of the winds changes from land–sea in the mornings to sea–land in the afternoons (Neetu *et al* 2006). The sampling stations experience the West India Coastal Currents (WICC) that brings in low saline, warm and nutrient depleted waters from the south (Shetye 1999; Naqvi *et al* 2003). Such hydrographic conditions support diatoms and dinoflagellates (Devassy and Goes 1989) which were also observed in this mixed phytoplankton bloom. Moreover, earlier reports suggest that diatoms (55%) and dinoflagellates (36%) constitute the phytoplankton population in the waters around CaTS (Parab *et al* 2006). The existing meteorological and hydrodynamic conditions suggest that the bloom might have developed off-shore and migrated to the coast over time, resulting in extremely high concentrations of Chl *a* in the surface waters.

This is the first report of a bloom in these waters wherein prasinoxanthin contribution was up to 17% of the diagnostic pigments. The waters in and around CaTS area is predominantly diatom based ecosystem with seasonal blooms of other phytoplankton species like *Trichodesmium* and a few dinoflagellates (Devassy and Goes 1988; Desa *et al* 2005) and prasinoxanthin pigment generally constitutes <5% of the pigment suite analyzed in these waters (Parab *et al* 2006). Our assessment of the mixed bloom is based on the combination of microscopic observations (table 2; figure 4) and measurements of marker pigments using HPLC (figure 3). Prasinoxanthin is a biomarker pigment for cells of class *Prasinophyceae* whereas Chl *b* appears in a range of phytoplankton groups including prasinophytes, euglenophytes and chlorophytes (Gibb *et al* 2001). All the microscopically identified phytoplankton species in this bloom do not contain prasinoxanthin and also do not contain endosymbionts that may have the marker pigment. Since the unidentified cells were the most abundant cells and the dinoflagellates identified in this bloom were not the source of prasinoxanthin, we believe that the presence of prasinoxanthin in the samples could be attributed to these cells. In the absence of clear taxonomic information using microscopy, we can only speculate that the unidentified algal cell may belong to class *Prasinophyceae*.

Table 3. *Blooms of non-indigenous species reported from west coast of India.*

No.	Bloom events	Dominant sp.	Bloom effect	Reference
1	Trichodesmium bloom	*Trichodesmium* sp.	Dark brown discolouration	Devassy *et al* (1978)
2	Dinoflagellate bloom	*Gymnodinium nagasakiense*	Red discolouration	Karunasagar and Karunasagar (1992)
3	Dinoflagellate bloom	*Noctiluca* sp.	Red discolouration & fish kill	Naqvi *et al* (1998)
4	Dinoflagellate bloom	*Noctiluca milaris*	Red discolouration	Nayak *et al* (2000)
5	Dinoflagellate bloom	*Noctiluca scintillans*	Red discolouration	Nayar *et al* (2001)
6	Trichodesmium bloom	*Trichodesmium* sp.	Dark brown discolouration	Sarangi *et al* (2004)
7	Dinoflagellate bloom	*Noctiluca milaris*	Red discolouration	Sahayak *et al* (2005)
8	Holoccolithophorid bloom	Unidentified holococcolithophore	Obnoxious stench	Ramaiah *et al* (2005)
9	Mixed phytoplankton bloom	Unidentified prasinoxanthin containing algal cells	Dirty brown discolouration	Present study

Pigment to Chl *a* ratios are widely used to estimate the relative contribution of different phytoplankton groups (Mackey *et al* 1996) and is a function of the physiological state of the cells, ambient nutrient levels and photoadaptive stress (Falkowski and LaRoche 1991). The Pras: Chl *a* (0.01–0.03) and Chl *b*: Chl *a* ratios (0.034–0.132) in the bloom samples were much lower than those reported in culture studies of prasinophytes (Pras: Chl *a* – 0.097 to 0.305; Chl *b*: Chl *a* – 0.618 to 1.313) (Latasa *et al* 2004). The lower pigment ratios in this study could be attributed to the age of the bloom, growth conditions and physiological stress. Another factor that may have skewed the pigment ratios in the present study was the contribution of Chl *a* from diverse phytoplankton population. A direct comparison of pigment ratios of a mixed population to monoculture-based studies is therefore highly subjective.

The age of the bloom could not be ascertained and contrary to the low pheaophytin *a* concentrations, the bloom had already entered the decline phase as evident from the decrease in Chl *a* concentrations over the sampling days (table 1) and microscopy (figure 4). The dominance of dinoflagellates and copepods in the bloom samples may have resulted in the low phaeophytin *a* concentrations. Accumulation of phaeophytin *a* differs with different grazers and need not always increase during the decline of a bloom. Engelkes (1985) found that copepod grazing did not form phaeophytin *a* from Chl *a* whereas protozoans could digest chlorophylls to colourless residues (Burkill *et al* 1987). Moreover, microzooplankton reingest fecal pellets resulting in extensive degradation of pigments and fast recycling of pigments within water column (Strom 1993).

The bloom was unusual for various reasons including exceptionally high concentration of Chl *a* by fluorometry, unique composition, presence of prasinoxanthin pigments and low biological activity. High Chl *a* concentrations by fluorometry was not reflected in HPLC method which may be due to combination of factors. Fluorometric analyses do not differentiate Chl *a* and Chl *b* and presence of phytoplankton groups with high amounts of Chl *b* can produce a significant fluorescence anomaly due to changes in the accessory pigment composition (Lorenzen and Jeffrey 1980; Trees *et al* 1985, 2000). Variations in extraction procedures can result in discrepancy of up to 87% between the two analytical techniques (van Heukelem 2002). Other sources of discrepancies include differences in sample collection, sample storage, nature of standards used for sample analysis, etc. Despite these discrepancies, HPLC pigment measurement is considered to be more reliable as it separate and quantifies various pigments and their derivatives (Jeffrey *et al* 1999).

This bloom was one-time event in these waters and has never been encountered previously (table 3). Subsequent sampling during the same month in following years did not show any exceptional Chl *a* values or phytoplankton composition. The stations along CaTS have been monitored continuously since mid 1990s and have been reported for its biogeochemistry (Naqvi *et al* 2003). Moreover, primary production and phytoplankton

communities in these waters have been monitored regularly since 1970s (Dehadrai and Bhargava 1972; Qasim 1977; Devassy and Goes 1989; Parab *et al* 2006). The occurrences of 'unusual' bloom events have been reported globally with increasing frequency. One of the common features of all the 'unusual' blooms in coastal waters is the dominance of non-indigenous species (NIS) that may reoccur sporadically with or without adverse effect (Cloern 1996). For example, nuisance brown tides in the northeast coast of United States have been attributed to sporadic bloom of *Aureococcus anophagefferens* (Bricelj and Lonsdale 1997). Similarly, intense blooms of toxic dinoflagellate and holococcolithophorids causing large scale fish kill and foul smell production in the southwest coast of India have been recorded periodically (Karunasagar and Karunasagar 1992; Karunasagar *et al* 1989, 1990; Ramaiah *et al* 2005). Unlike toxic blooms, non-toxic bloom of a prymnesiophyte *Phaeocystis globosa* was first reported in the Arabian Sea by Madhupratap *et al* (2000). Nevertheless, non-toxic blooms of NIS generally go unreported due to lack of adequate attention and continuous monitoring of the coastal waters.

The presence of prasinoxanthin in the bloom samples raises questions about its origin. Prasinoxanthin pigments in bloom samples are generally limited to higher latitudes (Reigman and Kraay 2001; Schluter and Møhlenberg 2003; Ansotegui *et al* 2003; Yu *et al* 2007). Nevertheless, cells having prasinoxanthin have been reported from Gulf of Mexico (Guillard *et al* 1991), tropical waters (Hallaegraeff and Jeffrey 1984; McManus and Dawson 1994) and equatorial waters (Mackey *et al* 1998; Gin *et al* 2003). Based on the archival data (table 3) and the nature of the bloom, we believe that the dominant phytoplankton cells were alien to these waters and were accidentally introduced. The sampling area is close to Mormugao Port, which is one of the major shipping ports along the west coast of India (figure 1), and experiences intense shipping activity largely with China, Korea and southeast Asian countries. The introduction of alien microalgal species in Indian waters is well established (Subba Rao 2005); nine dinoflagellate species and one diatom species belonging to the coastal waters of China, Japan, Mediterranean, western Atlantic and Australia have been reportedly introduced in and around Indian waters in the last 50 years. With increased maritime activity in and around the sampling waters, it is likely that this non-native phytoplankton could have been transported via ballast waters of the ships and released into the sampling station.

Blooms of non-indigenous species are sporadic and depend upon the adaptability of these forms to new environment and avoid potential predators/consumers. Apart from possible toxic/harmful effects, there is little information on the impacts of such blooms on the organic carbon composition and the stress on existing microbial food-web and associated biological communities. During the unusual bloom at CaTS, phytoplankton carbon (Chl *a*-C) estimated using OC:Chl *a* of 80 (Banse 1977) ranged from 8.5 g C m^{-3} at G1 (surface) to 56 mg C m^{-3} at G3 (14 m) on the first day sampling. Some of our values were much higher than those reported which could be attributed to the Chl *a* based carbon calculation adopted and under estimation of total organic carbon produced since dinoflagellates produce significant fraction of organic carbon as mucus exudates (figure 4b). The low microbial activity might be a reflection of the nature of organic matter produced during the bloom. Similar microbial response to organic carbon has been reported for a *Phaeocystis* bloom (Janse *et al* 2001). The low microbial activity and zooplankton biomass suggested that significant fraction of carbon fixed during the bloom escaped the food-web. The eventual sedimentation of such blooms and the shallow depth of the sampling stations would have increased organic carbon supply to the benthic communities that may influence their biomass and fecundity.

The ever increasing maritime activities around Indian waters raises concern about the vulnerability of the region to NIS through ballast water. It is very likely that similar blooms might be occurring along the Indian coastline but go largely unnoticed for want of continuous monitoring, awareness and effective mitigation measures both at policy formulation and implementation levels. Under Global Ballast Water Management Program of International Maritime Organization (IMO), extensive research on various aspects including baseline biota data, risk scenario development and analysis, GIS based database, development of demonstrable technologies for collection, supply and treatment of ballast water, etc. has been initiated at selected ports around the Indian coastline from 2001 (http://www.bwmindia.com/). These initiatives need to be further strengthened by proper documentation of such events and identification of NIS and related harmful events combined with effective coastal management tools is the need of the hour.

5. Conclusions

An unusual phytoplankton bloom dominated by unidentified cells and prasinoxanthin as one of the dominant pigments was encountered in a diatom-dominated marine environment in the near-shore waters off Goa. Based on the timing of the bloom,

morphology and pigment composition, we believe that this bloom was caused by a non-indigenous species which might have been introduced via ballast water discharge. The accumulation of bloom cells and dinoflagellates in a small patch in shallow waters combined with low bacterial production and zooplankton biomass resulted in unusually high concentrations of organic carbon. Although no immediate harmful impact of the bloom was evident, further studies are required to ascertain the impact of similar blooms on the microbial population, microbial loop and benthic fauna.

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